



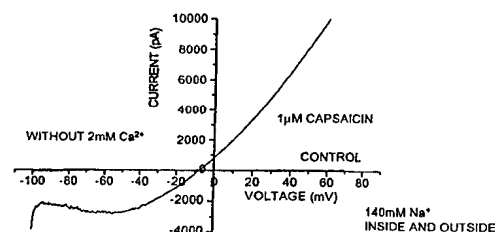
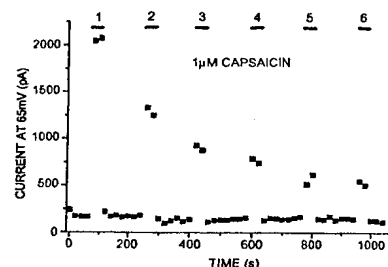
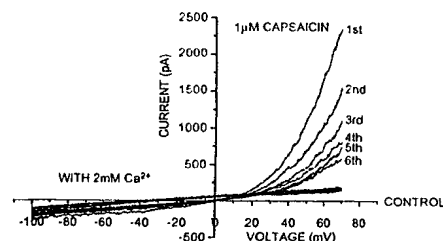
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(54) Title: HUMAN VANILLOID RECEPTORS AND THEIR USES

(57) Abstract

The invention provides novel human vanilloid receptor (hVR) proteins, in particular hVR1 and hVR3, nucleotide sequences encoding for the novel hVR proteins, and hVR proteins for use in a method for screening for agents useful in the treatment or prophylaxis of disorders which are responsive to modulation of hVR activity in a human patient. The invention also provides expression vectors comprising said nucleotide sequences, stable cell lines comprising said expression vectors, antibodies specific for the novel hVR proteins, methods for the identification of compounds which exhibit hVR modulating activity, compounds identifiable and identified by such methods, and methods of treatment or prophylaxis of disorders which are responsive to modulation of hVR activity in a human patient.



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HUMAN VANILLOID RECEPTORS AND THEIR USES

Field of the Invention

5 The present invention relates to human vanilloid receptor (hVR) proteins and to related nucleotide sequences, expression vectors, cell lines, antibodies screening methods, compounds, methods of production and methods of treatment, as well as other related aspects.

Background of the Invention

10 Capsaicin, the irritant in hot peppers and a member of the vanilloid family activates a sub-group of sensory neurons: the nociceptors. These neurons transmit nociceptive and thermoceptive pain information back to pain-processing centres in the central nervous system such as the spinal cord and the brain. They are also sites for the release of pro-inflammatory mediators in the
15 periphery (1). Nociceptors show heterogeneity in their sensitivity to capsaicin. Excitation and prolonged exposure of these neurons to capsaicin is followed by a refractory state known as desensitisation (2) when they become insensitive to capsaicin and other noxious stimuli (3). The long-term response to insensitivity could be explained by death of the nociceptors or destruction of its peripheral
20 terminals (4). Because of the desensitisation phenomenon, capsaicin has been used therapeutically for decades as an analgesic agent for the treatment of pain in a range of disorders (5).

25 It has been speculated that the endogenous target for capsaicin plays an important function in the detection of painful stimuli. It has been shown by electrophysiological and biochemical studies that capsaicin induces a flux of cations in dorsal root ganglion (DRG) neurons (6,7). Because other vanilloid derivatives show responses in a dose dependent manner (8,9) a receptor is the most likely candidate to explain the mechanism. Therefore, based on indirect
30 evidence it has been anticipated that these actions of capsaicin (excitation / desensitisation) are mediated by a specific membrane-bound receptor named vanilloid receptor (10).

35 Evidence for the existence of a vanilloid receptor came from binding experiments with resiniferatoxin (RTX), a capsaicin analog (11), and a competitive antagonist

of capsaicin, capsazepine (12). Vanilloid receptors have been visualised by using ($[^3\text{H}]$ -RTX) autoradiography in dorsal root ganglia (DRG) and spinal cord of different species including man (13,14).

5 Recently, a rat vanilloid receptor termed VR1 has been identified using an expression-cloning strategy to isolate the complementary DNA (cDNA) encoding the corresponding protein from a rat DRG cDNA library (15). The cDNA clone was completely sequenced. The rat VR1 cDNA has an open reading frame of 2,514 nucleotides and encodes for a protein of 838 amino acids with a predicted
10 relative molecular mass of 95,000. Analysis of the amino acid sequence identified 6 potential transmembrane regions with a short hydrophobic stretch between the transmembrane regions 5 and 6. The N-terminus (amino terminal) contains three ankyrin repeat domains. No motifs have been identified at the C-terminus (carboxy terminal).

15 It has been noted that rat VR1 transfected cells exhibit an increase in calcium levels after heat treatment and it has been suggested that *in vivo* VR1 and vanilloid receptors are involved in detection of noxious heat (but not innocuous heat). It has also been proposed that protons could act as modulators of the
20 vanilloid receptors (16, 17, 18).

While it has been recognised that the rat capsaicin receptor, VR1, is a member of the family of non-selective ion channels that are gated by ligands and that it is involved in pain sensation, the natural ligand of VR1 remains unknown. It is
25 therefore suggested that human vanilloid receptor sub-types may provide targets for the development of novel analgesic agents (agonists and antagonists) and agents which may interact with other disorders.

30 Accordingly, it is an object of the present invention to locate and characterise human vanilloid receptors. Other objects of the present invention will become apparent from the following detailed description thereof.

Summary of the Invention

35 According to one embodiment of the present invention there is provided an isolated human vanilloid receptor (hVR) protein or a variant thereof. Preferably

the hVR protein is an hVR1 or hVR3 protein or a variant thereof. In a particularly preferred aspect of the invention the hVR protein has an amino acid sequence as shown in figure 3 or in figure 18.

5 According to another aspect of the invention, there is provided a human vanilloid receptor (hVR) protein or a variant thereof, preferably hVR1 or hVR3 or a variant thereof, for use in a method of screening for agents useful in the treatment or prophylaxis of a disorder which is responsive to the modulation of hVR activity, preferably hVR1 or hVR3 activity, in a human patient. Preferably the disorder is
10 pain, neuropathic pain, inflammatory pain, chronic pain, post-operative pain, rheumatoid arthritic pain, neuropathies, neuralgia, algesia, neurodegeneration, nerve injury, stroke, ischaemia migraine, irritable bowel syndrome (IBS), a respiratory disorder such as asthma or chronic obstructive pulmonary disease (COPD), a urological disorder such as diabetic neuropathy, incontinence and
15 interstitial cystitis, or an inflammatory disorder.

According to another aspect of the invention there is provided a nucleotide sequence encoding a human vanilloid receptor (hVR) protein or a variant thereof as hereinbefore described, or a nucleotide sequence that is complementary thereto. Preferably the nucleotide sequence encodes an hVR1, hVR3 protein or
20 variant thereof or a nucleotide sequence which is complementary thereto. Particularly preferably the nucleotide sequence is as shown in figure 2 and figure 17.

25 According to another aspect of the invention there is provided an expression vector comprising a nucleic acid sequence as referred to above which is capable of expressing an hVR protein as hereinbefore described or a variant thereof, preferably hVR1 or hVR3 or a variant thereof. Preferably the expression vector is as displayed in figure 6 or figure 20.

30 According to another aspect of the invention there is provided a stable cell line comprising an expression vector as referred to above which is capable of expressing an hVR protein as hereinbefore described or a variant thereof, preferably hVR1 or hVR3 or a variant thereof. The stable cell line is preferably a

modified mammalian cell line, preferably HEK293, CHO, COS, HeLa or BHK although transient expression may be preferred in *Xenopus* oocytes.

5 According to another aspect of the invention there is provided an antibody specific for an hVR protein as hereinbefore described or a variant thereof, preferably specific for hVR1 or hVR3 or a variant thereof.

10 According to another aspect of the invention there is provided a method for identification of a compound which exhibits hVR modulating activity, comprising contacting an hVR protein as hereinbefore described or a variant thereof, preferably hVR1 or hVR3 or a variant thereof, with a test compound and detecting modulating activity or inactivity.

15 According to another aspect of the invention there is provided a compound which modulates hVR activity, preferably that of hVR1 or hVR3, identifiable by the method referred to above.

20 According to another aspect of the invention there is provided a compound which modulates hVR activity, preferably that of hVR1 or hVR3, identifiable by the method referred to above, for use in therapy.

25 According to another aspect of the invention there is provided the use of a compound which modulates hVR activity, preferably that of hVR1 or hVR3, identifiable by the method referred to above, in the manufacture of a medicament for treatment or prophylaxis of a disorder which is responsive to the modulation of hVR activity, preferably hVR1 activity or hVR3 activity, in a human patient. Preferably the disorder is pain, neuropathic pain, inflammatory pain, chronic pain, post-operative pain, rheumatoid arthritic pain, neuropathies, neuralgia, algesia, neurodegeneration, nerve injury, stroke, ischaemia migraine, irritable bowl syndrome (IBS), a respiratory disorder such as asthma or chronic obstructive pulmonary disease (COPD), a urological disorder such as neuropathy, incontinence or interstitial cystitis, or an inflammatory disorder.

35 According to another aspect of the invention there is provided a method of treatment or prophylaxis of a disorder which is responsive to modulation of hVR,

preferably hVR1 or hVR3, activity in a human patient which comprises administering to said patient an effective amount of a compound identifiable by the method referred to above. Preferably the disorder is pain, neuropathic pain, inflammatory pain, chronic pain, post-operative pain, rheumatoid arthritic pain, neuropathies, neuralgia, algesia, neurodegeneration, nerve injury, stroke, ischaemia migraine, irritable bowel syndrome (IBS), respiratory disorders such as asthma and chronic obstructive pulmonary disease (COPD) and urological disorders including diabetic neuropathy, incontinence and interstitial cystitis and inflammatory disorders.

According to another aspect of the invention there is provided a compound which modulates hVR activity, preferably that of hVR1 or hVR3, identifiable by the method referred to above, excluding the compounds capsaicin, resiniferatoxin, piperine, zingerone, polydodial, warburganal, aframodial, cinnamodial, cinnamosmolide, cinnamolide, isovelleral, scalaradial, ancistrodial, β -acaridial, scutigeral, merulidial, anandamide and capsazepine.

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According to another aspect of the invention there is provided the use of a compound which modulates hVR activity, preferably that of hVR1 or hVR3, identifiable by the method referred to above, excluding the compounds capsaicin, resiniferatoxin, piperine, zingerone, polydodial, warburganal, aframodial, cinnamodial, cinnamosmolide, cinnamolide, isovelleral, scalaradial, ancistrodial, β -acaridial, scutigeral, merulidial, anandamide and capsazepine, in the manufacture of a medicament for treatment or prophylaxis of a disorder which is responsive to the modulation of hVR activity, preferably hVR1 activity or hVR3 activity, in a human patient. Preferably the disorder is pain, neuropathic pain, inflammatory pain, chronic pain, post-operative pain, rheumatoid arthritic

pain, neuropathies, neuralgia, algesia, neurodegeneration, nerve injury, stroke, ischaemia migraine, irritable bowel syndrome (IBS), a respiratory disorder such as asthma or chronic obstructive pulmonary disease (COPD), a urological disorder such as neuropathy, incontinence or interstitial cystitis, or an inflammatory disorder.

According to another aspect of the invention there is provided a method of treatment or prophylaxis of a disorder which is responsive to modulation of hVR, preferably hVR1 or hVR3, activity in a human patient which comprises administering to said patient an effective amount of a compound identifiable by the method referred to above, excluding the compounds capsaicin, resiniferatoxin, piperine, zingerone, polydodial, warburganal, aframodial, cinnamodial, cinnamosmolide, cinnamolide, isovelleral, scalaradial, ancistrodial, β -acaridial, scutigeral, merulidial, anandamide and capsazepine. Preferably the disorder is pain, neuropathic pain, inflammatory pain, chronic pain, post-operative pain, rheumatoid arthritic pain, neuropathies, neuralgia, algesia, neurodegeneration, nerve injury, stroke, ischaemia migraine, irritable bowel syndrome (IBS), respiratory disorders such as asthma and chronic obstructive pulmonary disease (COPD) and urological disorders including diabetic neuropathy, incontinence and interstitial cystitis and inflammatory disorders.

According to another aspect of the invention there is provided a compound identified by the method referred to above.

According to another aspect of the invention there is provided a compound identified by the method referred to above, for use in therapy.

According to another aspect of the invention there is provided the use of a compound identified by the method referred to above in the manufacture of a medicament for treatment or prophylaxis of a disorder which is responsive to the modulation of hVR activity, preferably hVR1 activity or hVR3 activity, in a human patient. Preferably the disorder is pain, neuropathic pain, inflammatory pain, chronic pain, post-operative pain, rheumatoid arthritic pain, neuropathies, neuralgia, algesia, neurodegeneration, nerve injury, stroke, ischaemia migraine, irritable bowel syndrome (IBS), a respiratory disorder such as asthma or chronic

obstructive pulmonary disease (COPD), a urological disorder such as neuropathy, incontinence or interstitial cystitis, or an inflammatory disorder.

According to another aspect of the invention there is provided a method of treatment or prophylaxis of a disorder which is responsive to modulation of hVR, preferably hVR1 or hVR3, activity in a human patient which comprises administering to said patient an effective amount of a compound identified by the method referred to above. Preferably the disorder is pain, neuropathic pain, inflammatory pain, chronic pain, post-operative pain, rheumatoid arthritic pain, neuropathies, neuralgia, algesia, neurodegeneration, nerve injury, stroke, ischaemia migraine, irritable bowel syndrome (IBS), respiratory disorders such as asthma and chronic obstructive pulmonary disease (COPD) and urological disorders including diabetic neuropathy, incontinence and interstitial cystitis and inflammatory disorders.

According to another aspect of the invention there is provided a method of producing an hVR protein as hereinbefore described or a variant thereof, preferably hVR1 or hVR3 or a variant thereof, comprising introducing into an appropriate cell line a suitable vector comprising a nucleotide sequence encoding for an hVR protein or a variant thereof, preferably hVR1 or hVR3 or a variant thereof, under conditions suitable for obtaining expression of the hVR protein or a variant thereof, preferably hVR1 or hVR3 or a variant thereof.

Brief Description of the figures

Figure 1 is an alignment of hVR1 *in silico* derived clusters with rat VR1.

Figure 2 displays the human VR1 nucleotide sequence including the 5'UTR (nt – 773 to nt 0), coding region (nt 1 to 2517) and 3'UTR (nt 2518 to nt 3560).

Figure 3 illustrates the nucleotide and encoded amino acid sequence of the human VR1 sequence.

Figure 4 depicts the amino acid sequence of the hVR1 gene, the shading denotes predicted trans-membrane regions (boxed) and the ankyrin binding domains (unboxed). The predicted phosphorylation sites are underlined.

Figure 5 is a comparison of the amino acid sequences of the rat (rVR1) and human (hVR1) vanilloid receptors.

Figure 6 illustrates constructs pBluescriptSK(+) (A) and pCIN5-new (B) with the full length hVR1 gene cloned via NotI and EcoRI restriction sites.

Figure 7 shows a Slot Blot hybridisation with hVR1 probe with positive labelling of both rat and human DRG mRNA.

5 Figure 8 displays a Western blot probed with anti-VR1 antibodies with the arrow indicating the VR1 specific protein.

Figure 9 shows localisation of VR1 in rat DRG tissue sections, the arrow points to VR1 expressing small diameter (<25µm) neurone cell bodies.

10 Figure 10 depicts the *in situ* localisation of VR1 in human DRG sections (A) and human skin (B).

Figure 11 illustrates the functional response to capsaicin and blockade by capsazepine (CPZ) (A) with the current voltage relationship plotted in (B) on human VR-1 channels, transiently expressed in HEK293T cells.

15 Figure 12 shows capsaicin-induced desensitisation of human VR-1 channels in the presence of 2mM external calcium (A), maximum current (65mV) against time (B) and current voltage relationship in the absence of Ca²⁺ (C).

Figure 13 shows the influx of calcium into transiently transfected HEK293T cells over a time course in the presence of agonist capsaicin, anandamide and resiniferatoxin in the absence (A, B, D and F) or presence (C, E, G) of the antagonist, capsezipine.

20 Figure 14 illustrates a graphical presentation the results shown in figure 13 examining the response of hVR1 transfected HEK293T cells over time before and after exposure to agonists: capsaicin, anandamide and resiniferatoxin in the absence (A, B, D and F) or presence (C, E, G) of the antagonist, capsezipine.

25 Figure 15 displays the proposed assay strategy to carry out drug screening.

Figure 16 displays an alignment of *in silico* derived hVR3 specific clusters with rat VR1.

Figure 17 depicts the hVR3 nucleotide sequence including the 5' UTR (nt -686 to nt 0) Coding region (nt1 to nt 2889), 3'UTR (nt 2890 to nt 3418).

30 Figure 18 shows the nucleotide and amino acid sequence of hVR3.

Figure 19 is of the amino acid sequence of hVR3, the shading denotes predicted trans-membrane regions (boxed) and the ankyrin binding domains (unboxed).

Figure 20 displays constructs pBluescriptSK(+) (A) and pCDNA3.1 (+) (B) with the full length hVR3 gene cloned via NotI and XhoI restriction sites.

Figure 21 illustrates a multiple comparison of the amino acid sequences of the rat VR1 and the human vanilloid receptors: hVR1, hVRL-1 and hVR3.

Figure 22 Northern Blot hybridisation with hVR3 probe with strong signals detected in trachea (A), prostate (B), placenta, kidney and pancreas (C).

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Detailed Description of the Invention

Throughout the present specification and the accompanying claims the words "comprise" and "include" and variations such as "comprises", "comprising", "includes" and "including" are to be interpreted inclusively. That is, these words are intended to convey the possible inclusion of other elements or integers not specifically recited, where the context allows.

10

As referred to above, the present invention relates to isolated human vanilloid receptor (hVR) proteins, and in particular to the human vanilloid receptors which will be termed respectively human vanilloid receptors 1 and 3 (hVR1, and hVR3), sequence information for which is provided in figures 2 (hVR1) and 17 (hVR3). In the context of this invention the term "isolated" is intended to convey that the receptor protein is not in its native state, insofar as it has been purified at least to some extent or has been synthetically produced, for example by recombinant methods. The term "isolated" therefore includes the possibility of the receptor protein being in combination with other biological or non-biological material, such as cells, suspensions of cells or cell fragments, proteins, peptides, organic or inorganic solvents, or other materials where appropriate, but excludes the situation where the receptor protein is in a state as found in nature.

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Routine methods, as further explained in the subsequent experimental section, can be employed to purify and/or synthesise the receptor proteins according to the invention. Such methods are well understood by persons skilled in the art, and include techniques such as those disclosed in Sambrook, J. *et al.* (28), the disclosure of which is included herein in its entirety by way of reference.

30

By the term "variant" what is meant throughout the specification and claims is that other peptides or proteins which retain the same essential character of the human vanilloid receptor proteins for which sequence information is provided, are also intended to be included within the scope of the invention. For example,

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other peptides or proteins with greater than about 80%, preferably at least 90% and particularly preferably at least 95% homology with the sequences provided are considered as variants of the receptor proteins. Such variants may include the deletion, modification or addition of single amino acids or groups of amino acids within the protein sequence, as long as the peptide maintains the biological functionality of a human vanilloid receptor. This biological functionality can of course be assessed by conducting binding studies with known vanilloid modulators such as capsaicin, capsazepine (12) and resiniferatoxin (11).

Human VR1 is preferentially expressed in human dorsal root ganglia (DRG) and relative to hVR3 has the highest sequence homology with the rat VR1. Therefore, hVR1 is likely to be the human orthologue to rat VR1. hVR3 is less similar to rat VR1 and is expressed in a wider range of tissues. Nucleotide sequence analysis of hVR1 reveals a 2517bp open reading frame which encodes an 839 amino acid protein (see figures 2, 3 and 4). This deduced hVR1 protein sequence is 86 % identical to the rat VR1 (15) and shares many of its characteristics such as 6 transmembrane regions with an hydrophobic stretch between transmembrane 5 and 6 and an N-terminus which contains 3 ankyrin repeat domains. Similarly hVR3 has an open reading frame of 2889bp open reading frame which encodes a 963 amino acid protein (see figures 17, 18 and 19). The deduced hVR3 protein is 46 % identical to rat VR1 and 44 % identical to hVR1 sharing many of VR1's characteristics such as 6 transmembrane regions with an hydrophobic stretch between transmembrane 5 and 6 and an N-terminus which contains 3 ankyrin repeat domains.

The invention also includes nucleotide sequences which encode for human vanilloid receptor proteins or variants thereof as well as nucleotide sequences which are complementary thereto. Preferably the nucleotide sequence is a DNA sequence and most preferably, a cDNA sequence. Preferably the proteins are hVR1, hVR3 or variants thereof. Such nucleotides can be isolated or synthesised according to methods well known in the art. See reference 28, the disclosure of which is included herein in its entirety by way of reference.

The present invention also includes expression vectors which comprise nucleotide sequences encoding for the hVR, preferably hVR1 or hVR3, receptor

proteins or variants thereof. A further aspect of the invention relates to an expression vector comprising nucleotide sequences encoding for hVR1 or hVR3 receptor proteins or variants thereof. Such expression vectors are routinely constructed in the art of molecular biology and may for example involve the use of plasmid DNA and appropriate initiators, promoters, enhancers and other elements, such as for example polyadenylation signals which may be necessary, and which are positioned in the correct orientation, in order to allow for protein expression. Suitable vectors for use in practicing the present invention include pBluescript (Stratagene), pCR-Script (Stratagene), pCR2.1-TOPO (Invitrogen), pCRII-TOPO (Invitrogen), pCR-Blunt (Invitrogen), with vectors such as pCIN (32) (available from Clontech as pIRES-neo), pCDNA 3.1 (Invitrogen) or pCIneo (Promega) required for mammalian expression. Appropriate methods can be effected by following protocols described in many standard laboratory manuals (28, 29).

The invention also includes cell lines which have been modified to express the novel receptor. Such cell lines include transient, or preferably stable higher eukaryotic cell lines, such as mammalian cells or insect cells, lower eukaryotic cells, such as yeast or prokaryotic cells such as bacterial cells. Particular examples of cells which have been modified by insertion of vectors encoding for the receptor proteins according to the invention include HEK293T cells and *Xenopus* oocytes. Preferably the cell line selected will be one which is not only stable, but also allows for mature glycosylation and cell surface expression of the inventive receptor. Representative examples of appropriate hosts include animal cells such as HEK293, CHO, COS, HeLa and BHK.

It is also possible for the receptors of the invention to be transiently expressed in a cell line or on a membrane, such as for example in a baculovirus expression system. Such systems, which are adapted to express the receptors according to the invention, are also included within the scope of the present invention.

In particular, the functional hVR protein may include hVR receptor proteins selected from hVR1 and hVR3 and thereof or even other hVR protein subtypes or splice variants which have not yet been identified.

According to another aspect, the present invention also relates to antibodies, preferably monoclonal antibodies, which have been raised by standard techniques and are specific for the receptor proteins or variants thereof according to the invention. Such antibodies could for example be useful in purification; isolation or screening involving immuno precipitation techniques and may be used as tools to further elucidate hVR, preferably hVR1 or hVR3, protein function, or indeed as therapeutic agents in their own right. Antibodies may also be raised against specific epitopes of the receptors according to the invention.

An important aspect of the present invention is the use of receptor proteins according to the invention in screening methods designed to identify compounds which act as receptor ligands and which may be useful to modulate receptor activity. In general terms, such screening methods will involve contacting the receptor protein concerned, preferably hVR1 or hVR3, with a test compound and then detecting modulation in the receptor activity, or indeed detecting receptor inactivity, which results. For further details on the screening strategy refer to figure 15. The present invention also includes within its scope those compounds which are identified as possessing useful hVR, preferably hVR1 or hVR3, modulation activity, by the screening methods referred to above. The screening methods comprehended by the invention are generally well known to persons skilled in the art. High throughput screens may include fluorescence based assays using the Fluorometric Imaging Plate Reader (FLIPR) with calcium sensitive dyes, and reporter gene assays using calcium sensitive photoproteins that emit light on the influx of calcium and can be detected using an Imaging system. Secondary screens may involve electrophysiological assays utilising patch clamp technology to identify small molecules, antibodies, peptides, proteins or other types of compounds that interact with hVR, preferably hVR1 or hVR3, to modulate activity. Tertiary screens may involve the study of modulators in well characterised rat and mouse models of pain. These models of pain include, but are not restricted to, intraplantar injection of inflammatory agents such as carageenan, formalin and complete freunds adjuvant (CFA). Models of neuropathic pain such as loose ligature of the sciatic nerve are also included. Other screens may involve the study of modulators in human volunteers subject to topically applied capsaicin.

Another aspect of the present invention is the use of compounds which have been identified by screening techniques referred to above in the treatment or prophylaxis of disorders which are responsive to modulation of hVR, preferably hVR1 or hVR3, receptor activity, in a human patient. By the term "modulation" what is meant is that there will be either agonism or antagonism at the receptor site which results from ligand binding of the compound at the receptor. By the term "modulation" what is meant is that there will be either agonism or antagonism at the receptor site which results from ligand binding of the compound at the receptor excluding the compounds capsaicin, resiniferatoxin, piperine, zingerone, polydodial, warburganal, aframodial, cinnamodial, cinnamosmolide, cinnamolide, isovelleral, scalaradial, ancistrodial, β -acariadial, scutigeral, merulidial, anandamide and capsazepine. hVR, preferably hVR1 and hVR3, proteins have been implicated in disorders of the central nervous system (CNS), gastrointestinal (GI) tract, lungs and bladder and therefore modulation of hVR, preferably hVR1 or hVR3, receptor activity in these tissues will result in a positive therapeutic outcome in relation to such disorders. In particular, the compounds which will be identified using the screening techniques according to the invention will have utility for treatment and/or prophylaxis of disorders such as pain, neuropathic pain, inflammatory pain, chronic pain, post-operative pain, rheumatoid arthritic pain, neuropathies, neuralgia, algesia, neurodegeneration, nerve injury, stroke, ischaemia migraine, IBS, respiratory disorders such as asthma and COPD, urological disorders including diabetic neuropathy, incontinence and interstitial cystitis, and inflammatory disorders. It is to be understood however, that the mention of such disorders is by way of example only, and is not intended to be limiting on the scope of the invention.

The compounds which are identified according to the screening methods outlined above may be formulated with standard pharmaceutically acceptable carriers and/or excipients as is routine in the pharmaceutical art, and as fully described in Remington's Pharmaceutical Sciences, Mack Publishing Company, Eastern Pennsylvania, 17th Ed, 1985, the disclosure of which is included herein in its entirety by way of reference.

The compounds may be administered via enteral or parenteral routes such as via oral, buccal, anal, pulmonary, intravenous, intraarterial, intramuscular, intraperitoneal, topical or other appropriate administration routes.

- 5 The present invention will be further explained, by way of examples, in the appended experimental section. Reference examples are provided.

Experimental details

10 **Reference Example A: Identification of related human ESTs (Expressed Sequence Tags) (19) to the rat VR1 sequence by *in silico* analysis**

15 The full-length rat VR1 amino acid sequence (15) was used as a query sequence using the tBlastn (20) alignment program to identify related human genes in the dbEST (21) and Incyte (Incyte Pharmaceuticals, Inc., 3174 Porter Drive, Palo Alto, California 94304, USA) databases. Several human ESTs were identified and those with similarities greater than 50% selected for further analysis. One of these ESTs was T12251 previously shown to have 68% amino-acid identity and 84% similarity over a region of 70 amino acids (15). Full-length
20 cloning and functional characterisation of the gene represented by this cluster has been completed (30). This gene was denoted hVRL-1 and encoded a protein of 764 amino acid protein that was 48 % identical to the rat VR1 protein. All human ESTs from both databases were clustered to identify overlapping identical ESTs belonging to the same transcript. The GCG package (Wisconsin
25 Package Version 9.0, Genetics Computer Group (GCG), Madison, Wisconsin) and a program developed in house termed ESTBlast (22) were used to build up these clusters. In total, forty-three ESTs derived from different tissue sources and both EST databases were clustered into ten groups, one of these clusters represented hVRL-1. The remaining nine clusters have been named hVRa, hVRb, hVRc, hVRd, hVRe, hVRf, hVRg, hVRh and hVRi. For each EST the
30 tissue source was assigned according to the annotations in the dbEST and Incyte databases. Since no obvious starting codon was present and the cluster sequences were shorter than the rat VR1 transcript none of these clusters were likely to represent a full-length vanilloid receptor transcript. Finally hVRg, hVRh and hVRi collapsed into a single contig. Sequence analysis has shown that
35

these cDNAs are likely to be chimeric. The 5' end has weak similarities with the rat VR1 gene but the 3' end is identical to a DNA binding protein. No more work was pursued with that transcript.

5 **Reference Example B: Isolation of the human orthologue to the rat VR1 gene (reference examples B1-B4):**

Reference Example B1: *In silico* assembly of human VR1

10 The consensus nucleotide sequences from the ten clusters were searched with the tBlastx program (20) against the rat VR1 sequences to identify the most likely open reading frames. Frame shifts were corrected when the sequence trace files were available. Each cluster was aligned against the rat VR1 amino-acid sequence according to the Blastx results. The Blastx alignment program
15 (20) was used to compare the full-length rat VR1 protein with the amino-acid sequences of the ten clusters. The three clusters with the highest homology, displayed in figure 1, were aligned with the rat VR1 gene.

20 Cluster hVRa shared a high homology (70% identity and 75% similarity over a stretch of 107 amino acids) with the 5' of the rat VR1 sequence but did not seem to have a potential start codon. It contained two ESTs (EST1 and EST2) derived from the same tissue, bladder, and from the same patient. These two ESTs were selected for further investigation since this cluster was the most 5', had high homology with rat VR1 and the bladder tissue could be contaminated with
25 sensory neurones. Both cDNA clones were ordered but only clone EST1 was received as EST2 failed the recovery procedure.

30 Cluster hVRb composed of two EST's (EST3 and EST4), with 89% identity and 92% similarity over 90 residues, showed the highest degree of homology to the rodent sequence. The overlap between both sequences was located towards the middle of the gene.

35 hVRc (EST5) also while having high homology (71% identity and 75% similarity over 65 residues) with rat VR1 was closely related to the C-terminus of the rat protein sequence.

Reference Example B2: Sequencing of clones

5 All DNA sequences were determined by automated DNA sequencing based on the dideoxy chain-termination method using the ABI 373A / 377 sequencers (Applied Biosystems). Sequence-specific primers were used with the 'Big-Dye' Terminator Cycle Sequencing kit (Applied Biosystems). The nucleotide sequence was analysed using programs from the University of Wisconsin Genetics Computer Group package.

10 More specifically when sequencing an EST clone, the following protocol was adopted. The EST1 clone was grown using standard procedures and DNA was isolated using Qiagen columns. SP6 (5' ATTTAGGTGACACTATAG) and T7 (5' TAATACGACTCACTATAGGG) primers flanking the cloning site were used to
15 sequence both ends. Plasmid DNA (0.6 pmol) was used with 10.0 pmol of each primer for the dye terminator reaction. The SP6 end corresponded to the *in silico* derived EST sequence (identical to EST1). The T7 end did not have homologies with VR1 nor did it possess a long open reading frame or a polyadenylation motif. The size of the insert was determined by enzyme digestion of the DNA
20 with the endonucleases NotI and EcoRI and calculated to be approximately 3kb.

Plasmid DNA (50ng) was used to amplify the insert by Polymerase Chain Reaction (PCR) with T7 and SP6 as primers. The PCR conditions included an
25 initial hot-start at 94°C for 2 minutes, followed by 35 cycles at 94°C for 45 seconds, 50°C for 45 seconds and 72°C for 1 minute and terminated by 5 minutes at 72°C. The resulting PCR amplicon was separated on a 1.2% agarose gel and shown to be of ~3kb in size.

30 To fully sequence the PCR product the nuclease-Bal-31 technique was used where both strands of duplex DNA are degraded from both ends (23). After ethanol precipitation of the PCR product, the pellet was re-suspended in 30ml of 1X Bal-31 buffer (add buffer composition). A time-course digest with 2 units of Bal-31 enzyme (Roche Molecular Biochemicals) was carried out with 12 time
35 points taken over 90 minutes (30 seconds, 1, 2, 3, 5, 7, 10, 15, 25, 45, 75 and 90 minutes). Three pools were made respectively from digests 1 to 4, 5 to 8 and 9

to 12. Each pool was blunt-ended and sub-cloned into the pCR-Script SK (+) plasmid from Stratagene at the SrfI site. After transformation, 16 colonies from each pool were screened by PCR with the flanking Reverse (5' GGAAACAGCTATGACCATG) and M13-20 (5' GTAAAACGACGGCCAGT) primers. The amplicons of 6 positive colonies per pool were subjected to direct sequencing (24) using the T3 (5' AATTAACCCTCACTAAAGGG) and T7 primers. The DNA sequences obtained were assembled using the GCG package, translated and aligned against the rat VR1 gene using the Blast tools. After analysis, the 3079bp amplicon was shown to have 2 introns of 603bp and 1221bp. The latter intron was located at the 3'end of the PCR product. The coding sequence covered 1255 bp and was separated by the former intron. Therefore the clone EST1 was likely to be a partially spliced and incomplete cDNA.

The clone belonging to cluster 1b (EST3) and derived from a kidney cDNA library was ordered and sequenced using the Bal-31 technique. After assembly of the sequences using the GCG package an identical overlap was identified with the DNA sequence of the cluster hVRc. Moreover a 3'end with a polyadenylation signal and tail was identified. The complete sequence of the combined hVRb Bal-31 derived sequence and hVRc was 2063 bp (1020 bp of coding and 1043 bp of 3' untranslated sequence).

Reference Example B3: Amplification of the middle section of hVR1 using the Polymerase Chain Reaction

We formulated the hypothesis that both sequences (hVRa and hVRb/c) were part of a common transcript. If the human and rat VR1 were going to be similar, the 2 contigs should be separated by a gap of approximately 275bp. Primers were designed on both sides of the gap to amplify mRNA from brain tissues in order to clone the gap. A smear was obtained with the sense primer (5' TCTACTTCGGTGAAGTCCCC) and antisense (5' ACGGCAGGGAGTCATTCTTC). For specificity 50ng of the PCR product were amplified with the nested sense (5' CTGCAGAACTCCTGGCAGA) and antisense (5' GTCACCACCGCTGTGGAAAA) primers. The 900bp nested amplicon was sequenced and shown to be identical to hVRa at one end and

hVRb/c at the other end. The middle part of the PCR product was homologous to the rat VR1 sequence. This region corresponded to 91 amino acids. When the sequences of hVRa, hVRb/hVRc and the internal amplicon are combined the total length of the Open Reading Frame (ORF) is 824 amino acids followed by a 3' untranslated sequence of 1043 bp. The human amino acid sequence is 87% identical to the rat sequence over that part of the coding region. This sequence was termed hVR1 because of its high degree of identity with the rat VR1 sequence.

Reference Example B4: Isolation of the 5' Terminus of hVR1 by PAC isolation

Since no start codon was identified at the 5' end an additional strategy was designed to identify the full-length sequence. Two primers, sense (5' TCCTCTGGCTTCCAACCCGTT) and antisense (5' GAACTGGGCAGAAAGTGCCT) were designed to amplify a 150bp product from the first intron mentioned in reference example B2. A P1 Artificial Chromosome (PAC) genomic clone (25) was isolated by PCR screening of a PAC library (Genome Systems, St Louis, Missouri). PAC DNA was recovered by using standard plasmid isolation protocol (26). An anti-sense primer was designed (5' CTGGAGTTAGGGTCTCCATCC) to sequence the genomic clone towards the potential 5' end of the gene. An open reading frame with a starting codon was identified. The gene structure was confirmed by using the GenScan software (27). The complete gene has a nucleotide sequence of 2517bp (figure 2) and encoded a 839 amino acid protein (Figures 3 and 4). The gene was named hVR1. Multiple alignment of the amino acid sequence of hVR1 and rat VR1 shows a remarkable degree of identity and similarities between both sequences (figure 5). The rVR1 and hVR1 amino acid sequences are 86% identical. Moreover after protein analysis 6 trans-membrane domains and 3 ankyrin binding domains were identified in hVR1 as in the rat VR1 gene.

Example 1: Full-length Amplification of hVR1 from human DRG and assembly into cloning vectors

HVR1 was PCR amplified in three sections from human DRG template. The 5' fragment was amplified using a sense primer encoding a NotI site and a strong

Kozak motif followed by gene specific sequence (5' GTCATAGCGGCCGCGCCGCCACCATGAAGAAATGGAGCAGCAC) and an antisense primer (5' AGGCCCACTCGGTGAACTTC). The thermo-cycling conditions used for this amplification included a hot start at 94°C for 4 mins, followed by 35 cycles of 94°C for 1 min, 54°C for 1 min and 72°C for 1 min. A final extension step of 72°C for 5 min completed the reaction. The resulting PCR products were separated on a 2% agarose gel and cloned into pCR[®]II-TOPO according to the manufacturers instructions supplied with the TOPO[™] TA Cloning[®] kit (Invitrogen). The middle section of hVR1 was PCR amplified using the sense primer: 5' GACGAGCATGTACAATGAGA and antisense primer: 5' GTCACCACCGCTGTGGAAAA. The cycling conditions included a hot start at 94°C for 4 mins, followed by 35 cycles of 1 min at 94°C, 56°C and 72°C. A final extension step of 72°C for 5 min completed the reaction. A band of approximately 870 bp was excised from a 2 % agarose gel and cloned as detailed by the TOPO[™] TA Cloning[®] kit into the vector pCR2.1[®]-TOPO. Finally the 3' end was PCR amplified with the sense primer: 5' TGTGGACAGCTACAGTGAGA and the antisense primer: 5'TGCACTGAATTCGAGCACTGGTGTTCCTCAG which encoded an EcoRI site for cloning. The PCR conditions included a 90 sec hot start at 94°C followed by 35 cycles of 94°C for 50 sec, 50°C for 50 sec and 72°C for 50 sec. The cycling was completed with a 72°C step for 5 min. PCR products were separated on a 2% agarose gel and cloned into the vector pCR2.1[®]-TOPO.

Resulting clones for each of the three hVR1-fragments were taken for sequence analysis and separate clones coding a consensus sequence were used in the full length assembly of the gene. The NotI/DraIII (New England Biolabs) digested 5' end fragment ligated together with the middle DraIII/EcoRI fragment into a NotI/EcoRI restricted pBluescript SK (+) vector (Stratagene). Finally, the remaining 3' fragment was introduced into the resulting construct via MscI and EcoRI restriction sites, a map of the resulting construct is displayed in figure 6A.

Several clones were selected for sequence analysis to confirm that constructs still encoded the hVR1 consensus sequence. These were then digested with NotI/EcoRI and ligated into the mammalian expression vector pCIN5-new (a modified version of pCIN1 (32) having an IVS deletion as well as a 36 bp

deletion repositioning the start codon of neomycin phosphotransferase immediately after the upstream EMVC IRES) as illustrated in figure 6B.

Example 2: Chromosomal Localisation

5 The primers used to isolate the PAC clone (reference example B4) were selected for PCR on the G3 radiation hybrid panel from Stanford commercially available from Research Genetics (Huntsville, Alabama). The positive lanes and negative patterns were analysed using the public web server at Stanford University (<http://www-sghc.stanford.edu>). After analysis the hVR1 gene
10 appears to be located on human chromosome 17 around marker SHGC-36073 (lod score=9.55).

Example 3: mRNA Distribution

15 The tissue distribution of hVR1 was established by slot-blot hybridisation. RNA was transferred onto a sheet of GeneScreen hybridisation transfer membrane (DUPONT) sandwiched in a slot blotter by suction via a vacuum pump. Once the membrane was rinsed in 2x SSC (3M sodium chloride and 0.3M sodium citrate pH7) for 2 min it was exposed to UV using an Ultraviolet crosslinker (Amersham Life Science) for 1min at 15000uW/cm² thus enabling cross-linkage of the RNA
20 onto the membrane. The amounts of RNA on the blot are unknown. The probe was obtained by PCR amplification of a 260 bp product of the coding region of hVR1 with the following two primers: 5' TGTGGACAGCTACAGTGAGA and 5' GTGGAAAACCCGAACAAGA. Membranes were hybridised for 4 hr shaking at 60°C in a 10% dextran sulphate, 1% SDS (sodium dodecyl sulphate) and 1M NaCl solution. The probe was labelled with [α 32P]dCTP (Amersham) using the RediprimeTM DNA labelling system (Amersham), so as to obtain approximately 500,000cpm of the labelled probe per ml of prehybridisation solution. Briefly
25 100ng of probe was boiled for 3 minutes (denaturization) and then cooled on ice for 2 minutes in a total volume of 45 μ l. This was added to the labelling tube from the kit together with 3 μ l of 32P dCTP followed by an incubation at 37°C for 30 minutes. 400 μ l of Herring Sperm DNA (Sigma) at a concentration of 8 μ g/ml was added to the labelled probe and heated at 99°C for 3 minutes followed by rapid cooling on ice. The labelled probe was added and mixed well in pre-hybridisation solution. The membranes were hybridised overnight at 55°C.
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The membranes were then washed, first at room temperature in 2xSSC and 1% SDS for 5 minutes, followed by 2x SSC and 1% SDS for 30 min at 50°C. If necessary further washes with 1x SSC and 0.5% SDS or 0.1xSSC and 0.1% for 30 mins at the same temperature were carried out. The membranes were then exposed to Scientific Imaging Film AR (Kodak) using intensifying screens at – 70°C overnight and the film developed.

The results are shown on figure 7. Strong signals were observed with the positive controls (slots 4B and 5B). Signals are detected on the human DRG slots (1A and 1B). No signals were detected with the water control (slot 3B). Three multi-tissue northern blots (Clontech) with a wide range of tissues have also been hybridised with the same probe, however no signals were detected. RT-PCR was performed on various tissues with the primer combination used to amplify the probe. A strong band was detected in DRG RNA. Taken together these hybridisations suggest that hVR1 is specifically expressed in neuronal tissue and DRG in particular.

Example 4: Design and production of Anti-hVR1 Antibody

The peptides CHFTTTRSRTRLFGKGDSEEASC (peptide68) and CGSLKPEDAEVFKDSMVPGEK (peptide69) were synthesised by standard solid phase techniques and purified by gel filtration chromatography. These peptides were conjugated via their Cys residues to the carrier protein, Tuberculin PPD (purified protein derivative) using sulpho-SMCC (sulfosuccinimidyl 4-[N-maleimidomethyl]-cyclohexan-1-carboxylate). Rabbits, previously sensitised to Bacillus Calmette Guerin (BCG), were inoculated with the resulting conjugates emulsified in incomplete Freund's adjuvant at approx monthly intervals. Serum was prepared from blood samples taken 7 days after each immunisation. The specific antibody response was followed by indirect enzyme-linked immunosorbent assay (ELISA) using free peptide as antigen. Immunoglobulins were purified from high titre sera using immobilised peptide affinity columns (sulpholink Pierce). Rabbits designated M143, 144 and 145 received peptide68 conjugate, rabbits M146, 147 and 148, peptide69 conjugate.

The antibodies have been validated by specific staining of the recombinant protein expressed in HEK293 cells. Whole cell lysates were prepared in Sample

Buffer (4 ml dH₂O, 1 ml 0.5 M Tris-HCl, pH 6.8, 0.8 ml glycerol, 1.6 ml 10 % w/v SDS, 0.4 ml 2-β mercaptoethanol and 0.2 ml of 0.05 % w/v bromophenol blue) and proteins separated by SDS-PAGE and transferred to a nitrocellulose filter by electroblotting. Following incubation with the antisera, bound immunoglobulins were revealed using HRP-conjugated secondary antibodies and enhanced chemiluminescence (ECL) detection. The antisera showed specific binding to a protein(s) of the appropriate molecular weight(s) in extracts of VR1 transfected cells, but not in control extracts, this is illustrated in figure 8.

Example 5: *In situ* localisation of hVR1 using specific antibody

The purified immunoglobulins have been used for immunohistochemical staining of rat DRG tissue sections. Fixed cryosections of DRG were incubated with antibodies for 48h at 4°C at concentrations between 0.1 to 0.5µg/ml. Following a washing step, bound antibodies were detected by indirect immunofluorescence. The antibodies recognised exclusively small diameter cell bodies of the peripheral sensory neurones as displayed in figure 9. This observation has been extended to human DRG tissues for the anti-peptide68 peptide antibodies demonstrating cross-reactivity with the human sequence as expected. Figure 10A demonstrates labelling of DRG cell bodies with an arrow that points to small diameter neuronal cell body) and in figure 10B the arrow points to labelled neurones innervating human skin.

Example 6: Mammalian Cell Expression (examples 6a-6b)

Example 6a: Transient expression of hVR1 in mammalian cells

HEK293 cells were plated onto a 6 well plate, containing poly-l-lysine coated coverslips, at 5×10^4 cells per well. Next day, fresh media was added to the cells (50% confluent). CalPhos Mammalian Transfection Protocol (Clontech, K2051-1) was used for DNA transfection. For each well of cells, solution A was made up containing 8ug hVR1pCIN5, 2µg pEYFP-N1 reporter DNA, 12.4 µl calcium solution and water to 100µl. Solution B (hepes buffered saline) was slowly vortexed while solution A was added dropwise. The mixture was incubated at room temperature for 20 minutes, and then added to cells. The plate was slowly rocked to distribute the solution. The cells were incubated at 37°C for 5 hours, and then washed with phosphate buffered saline. Fresh culture

medium was added and the plate was incubated 24-48 hours for functional analysis.

Example 6b: Stable expression of hVR1 in mammalian cells

5 HEK293 cells were plated onto a 6 well plate at 1×10^5 cells per well. Next day, fresh media was added to the cells (50% confluent). CalPhos Mammalian Transfection Protocol (Clontech, K2051-1) was used for DNA transfection. For each well of cells, solution A was made up containing 2 μ g hVR1pCIN5, 12.4 μ l 2M calcium solution and water to 100 μ l. Solution B (hepes buffered saline) was
10 slowly vortexed while solution A was added dropwise. The mixture was incubated at room temperature for 20 minutes, and then added to cells. The plate was slowly rocked to distribute the solution. The cells were incubated at 37°C for 5 hours, and then washed with phosphate buffered saline. Fresh culture medium was added and the plate was incubated 48 hours at 37°C, 5% CO₂.
15 Cells were harvested into 100mm dishes in selection medium containing 800 μ g/ml geneticin. Cells were then incubated and fed at 4 day intervals. In total around 10 days selection is required for each single cell to multiply into a visible clone. Well-separated clones were each picked (with a gilson tip) into separate wells of a 96 well plate, containing maintenance medium (400 μ g/ml geneticin).
20 Cells were expanded into flasks for freezing stocks and functional analysis. Stable cells may be plated at 1×10^5 cells onto poly-L-lysine coated coverslips in 6 well plate, for calcium imaging next day.

Example 7: Functional Analysis of hVR1(examples 7a-7c):

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Example 7a: Electrophysiology using patch clamp methods

The activation of human VR-1 channels transiently expressed in HEK293T cells by capsaicin was investigated. Cells grown on poly-L-lysine-coated glass coverslips were placed in a recording chamber (0.5ml) and superfused with
30 extracellular solution (2ml min⁻¹). The extracellular solution contained: NaCl (140mM), KCl (5mM), MgCl₂ (2mM), CaCl₂ (2mM), 4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid (HEPES, 10mM) and glucose (10mM). The pH was adjusted to 7.4 with NaOH and osmolarity ranged from 310-320mOsm l⁻¹. Patch pipettes (borosilicate glass) were pulled using a Sutter P-97 electrode
35 puller. The pipettes were filled with an internal solution consisting of: CsCl

(140mM), ethylene glycol-bis(β -aminoethyl ether) *N,N,N',N'*-tetra acetic acid Cs salt (Cs-EGTA, 5mM) and HEPES (10mM). The pH was adjusted to 7.25 using CsOH and the osmolarity ranged from 275-290 mOsm. When filled with this internal solution, patch electrodes had resistances of 2-5 M Ω . Currents were recorded using standard whole-cell voltage clamp recording techniques (31) at room temperature (21-23°C) using an Axopatch 200A amplifier and signals were sampled at 2 or 0.1 kHz. The majority of series resistance errors (80-85%) were minimized with compensation circuitry. Membrane potentials were not corrected for junction potentials (<4 mV). Voltage pulses and data collection were performed on-line using pClamp8 software (Axon Instruments) interfaced with amplifiers. Membrane potentials were maintained at -60mV between protocols.

Capsaicin or capsazepine (CPZ) were applied, using a 'fast-flow sytem', directly onto the recording cell (<1s to equilibrate). The effects of capsaicin were measured either by application during constant recording while holding the membrane potential at -60mV to elicit an inward current, or applying voltage ramps (-100 to +60mV) in the absence and presence of capsaicin. Similarly both these methods of recording currents evoked by the application of capsaicin were used to demonstrate the blockade by the antagonist (CPZ).

Figure 11A reveals that application of capsaicin (1 μ M), on human VR1 channels transiently expressed in HEK293T cells, produces an inward current when the membrane was held at a potential of -60mV. This response was abolished by 1 μ M CPZ and the blockade was partially reversible.

In the presence of 1 μ M capsaicin, voltage ramps (-100 to +70mV) produced a current-voltage relationship demonstrating a substantial outward rectification. Addition of 1 μ M CPZ completely blocked the current (figure 11B). Again, only partial recovery was observed, especially for the inward currents evoked by negative potentials.

Capsaicin-induced desensitisation of human VR-1 channels in the presence of 2mM external calcium is illustrated in figure 12. Voltage ramps (-100 to +70) were applied and the addition of capsaicin (1 μ M) evoked an outwardly rectifying current. Repeated additions of capsaicin resulted in a progressive 'rundown' in

the size of the response (figure 12A). Figure 12B shows a plot of the current elicited at a potential of +65mV against time illustrating the 'rundown' in current amplitude. Voltage ramps were applied every 20s and capsaicin added at 2min intervals for approximately 40s. By the 6th addition the current had reduced about 4-fold.

When the external calcium was replaced with 5mM EGTA the size of the current increased dramatically (figure 12C). However, when calcium was re-applied to the external solution, the current evoked by capsaicin (1 μ M) was approximately equivalent to that of the 6th addition shown in (figure 12A).

Example 7b: Calcium Imaging with HEK293 expressing hVR1

HEK293 cells expressing hVR1 transiently or stably, were plated onto poly-l-lysine coated cover slips at 1×10^5 cells per well. They were analysed on the following day by calcium imaging (QuantiCell 700, Applied Imaging). On the day of experiment, WASH buffer was prepared by adding CaCl_2 to extracellular medium (ECM) to a final concentration of 2mM, (ECM contains 125mM NaCl, 5mM KCl, 2mM MgCl_2 , 0.5mM NaH_2PO_4 , 5mM NaHCO_3 , 10mM Hepes, 10mM glucose, 0.1% BSA, pH7.4). The calcium sensitive dye solution was prepared by adding 50 μ l 5% pluronic F-127 in DMSO (Molecular Probes) to a vial of fura2-AM (Molecular Probes). After mixing, 20 μ l of the fura2-AM solution was added to 10ml WASH. 1.5 ml was then added to cells, which were then incubated at 37°C for 30 minutes. The plate was washed three times with WASH. 1ml WASH was added and stored in dark. Agonists and antagonists were prepared in WASH at 5x their required assay concentrations. The reagents and assay temperature was kept at 37°C. For the transiently transfected cells, the YFP reporter DNA fluorescence (490nm excitation) was used to identify the transfected cells. Cells were initially imaged in 400 μ l WASH (or 300 μ l WASH plus 100 μ l antagonist e.g. capsazepine). After approximately 1 min, 100 μ l agonist (e.g. capsaicin, anadamide or resiniferatoxin) at 5 x the desired concentration was added to give final 1x concentration. A sequence of images (340/380nm excitation) were taken to monitor calcium influx response in cells before (30-60 secs), and after the addition of agonist (2-5 mins). Figure 13 displays time courses taken for each of the tests set up to look at the affect of the different agonists mentioned above in the presence or absence of the rat VR1 antagonist, capsazepine. The Imager

also plots graphs of respective calcium concentration (nM) versus time (seconds) as shown in figure 14. After the addition of agonist (e.g. capsaicin, indicated by the vertical arrow on graph), the cells expressing hVR1 are stimulated to influx calcium. This is shown by the appearance of peak on the trace. The peak height correlates with hVR1 expression level. Varying levels of expression is some times seen depending on which cells are selected for the graph. Similar experiments may be accomplished to examine the response of protons and heat.

Example 7c: Use of a FLIPR assay with VR1

FLIPR (Fluorometric Imaging Plate Reader) is a high throughput fluorescence-based drug discovery tool for functional cell analysis. Intracellular calcium is monitored with the calcium sensitive dye, fluo3-AM. HEK293 cells stably expressing rat VR1 were plated into a 96 well, poly-l-lysine treated FLIPR plate at 3×10^4 cells per well. On the following day, the plate was processed for FLIPR. FBP buffer was prepared (15 μ M Probenecid (calcium ATPase pump blocker) in 1x FLIPR buffer (145mM NaCl, 5mM KCl, 1mM MgCl₂, 2mM CaCl₂, 10mM glucose, 20mM Hepes). FBP buffer pH was then adjusted to 7.4 with NaOH. 400 μ l DMSO was added to a vial of fluo3-AM (Cambridge Bioscience, F-1241). The fluo3-AM solution was incubated at 37°C for 10 min and vortexed. LOAD was prepared by adding 20 μ l of fluo3-AM solution and 20 μ l 20% pleuronic F-127 in DMSO (Cambridge Bioscience, P-3000) into 10 ml FBP. The 96 well plate containing cells was flicked off to remove cell medium. 100 μ l LOAD was added per well. Cells were then incubated at 37°C for 60 minutes. Capsaicin (a rVR1 agonist) and capsazepine (CPZ, a rVR1 antagonist) were prepared at 10x the desired final assay concentrations in FBP. The plate was flicked to remove LOAD from cells, and 180 μ l FBP was added per well. The FLIPR machine added 20 μ l capsaicin per well to give a final 1x concentration. Cells were monitored for 70 seconds after agonist addition. The FLIPR traces (fluorescence change (counts) versus time (seconds)) were produced for each well. Peaks indicate capsaicin-gated calcium influx, by cells expressing rVR1. The peak height correlates with the rVR1 expression level. To measure antagonism of the VR1 response 20 μ l 10x antagonist CPZ was added into wells to give a final 1x concentration. The plate was incubated for 15 minutes at room temperature prior reading in the FLIPR. The FLIPR traces recorded for each well show that the

peak heights are reduced in cells pre-incubated in CPZ. The same FLIPR assay may be used to monitor the response of human VR1 on exposure to agonists and antagonists.

5 **Example 8: Example of a screen using human VR1.**

FLIPR assay technology may be utilised to screen for hVR1 modulators according to the procedure described in figure 15. Human VR1 may be gated with protons, capsaicin or heat.

10 **Reference Example C: Identification and partial characterisation of additional human vanilloid receptors (reference examples C1-C3):**

Reference Example C1: Identification and characterisation of a novel vanilloid-like receptor, hVR3

15 ESTs belonging to the remaining clusters were characterised by *in silico* cloning (reference example A). The following clones were used during this process: - EST6/EST7 (hVRd), -EST8. (hVRe), - EST9/EST10. (hVRf). These EST clusters have been aligned with rat VR1 in figure 16, note that this diagram is not to scale.

20

Reference Example C2: Sequencing of clones

 Further sequencing, as detailed in reference example B2, and *in silico* cloning, enabled clusters hVRd, hVRe and hVRf to collapse forming a single contig of 583 amino acids. This sequence was named hVR3 and has 49 % identity with
25 the rat VR1 sequence. It was unlikely that this single contig was a full-length vanilloid receptor transcript as no obvious starting codon was present and it was shorter than the rat VR1 transcript.

Reference Example C3: Identification of the 5' terminus of hVR3

30 Two primers (sense primer 5' ATGGCCACCAGCAGGGTTAC and antisense primer 5' TCTGCCAGGTTCCAGCTG) designed to PCR amplify an amplicon stretching the 3' end of hVR3 and its 3'utr were used to isolate a genomic PAC clone (Genome Systems. St Louis, Missouri). The hVR3 specific PAC clone was then used as template to generate a library. This was achieved by sonicating
35 6µg of Qiagen purified PAC construct, size selecting fragmented DNA of 500-

2000bp. These resulting fragments were then blunt ended and cloned into the vector pCR®-Blunt as detailed in the manufacturers protocol supplied with the Zero Blunt™ PCR cloning kit (Invitrogen). Clones were then sequenced (reference example B2) to identify the complete 5' end of the hVR3 transcript.

5 The full-length nucleotide sequence of the hVR3 gene is displayed in figure 17. Figure 18 illustrates both nucleotide and encoded amino acid sequence of the human VR1 and figure 19 depicts the amino acid sequence of the hVR3 gene with shaded regions denoting predicted trans-membrane regions (boxed) and the ankyrin binding domains (unboxed).

Example 9: Full-length Amplification of hVR3 from human kidney template

Human kidney was used as a source of template for the PCR amplification of hVR3. Primers used for amplification were designed to isolate the gene in three fragments. Primers designed to isolate the 5' end included a sense primer encoding a NotI site and a strong Kozak motif followed by gene specific sequence (5' GTCATAGCGGCCGCGCGCCACCATGCCCAGGGTAGTTGGAC and antisense primer (5' CACCTCTTGTTGTCACTGGA). The PCR conditions used were a hot start at 94°C for 4 mins, followed by 35 cycles of 94°C for 1 min, 56°C for 1 min and 72°C for 1 min and finally one cycle at 72°C for 5 min. The resulting PCR products were separated on a 2% agarose gel and cloned into pCR®II-TOPO according to the manufacturers instructions supplied with the TOPO™ TA Cloning® kit (Invitrogen). The middle fragment was PCR generated using sense and antisense primers 5' CAAATCTGCGCATGAAGTTCCAG and 5' GCCACGAGAAGTTCCACGTAGTG respectively in the presence of 5% DMSO.

20 The PCR thermo-cycling required 35 cycles of 1 min at 94°C, 58°C and 72°C for successful amplification of the fragment which was then excised from a 2% agarose gel for cloning into the pCRII®-TOPO vector. Finally the 3' fragment was amplified with a sense primer 5' GCTGCTCCCATTCTTGCTGA and an antisense primer 5' TGCACTCTCGAGAAATGAGTGGGCAGAGAAGC encoding a XhoI restriction site. This fragment was successfully amplified using a hot start at 94°C for 4 min followed by 35 cycles of 94°C for 50 sec, 48°C for 50 sec and 72°C for 2 min. The cycling was completed with a 72°C step for 5 min. The amplified fragment was excised from a 2% agarose gel and clone into the pCRII®-TOPO vector.

Resulting clones for each of the three PCR generated hVR3-fragments were taken for sequence analysis and separate clones coding a consensus sequence were used in the full-length assembly of the gene. The DraIII restriction site of the pBluescript SK (+) vector (Stratagene) was firstly abolished by digestion with
5 DraIII followed by a blunt ending step using T₄ DNA polymerase (New England Biolabs). This modified vector was then restricted to enable the ligation of both a NotI/NcoI 5' fragment and NcoI/ EcoRI middle fragment. Finally, the remaining 3' fragment was introduced into the resulting construct via DraIII and XhoI sites (figure 20A).

10 Several clones were selected for sequence analysis to confirm that the constructs still encoded the hVR3 consensus sequence. These were then digested with NotI/XhoI and ligated into the mammalian expression vector pCDNA3.1 (+) (Invitrogen) as seen in figure 20B. The resulting hVR3 consensus
15 sequence is shown in the multiple alignment along with the full-length sequence of hVR1 and the published hVRL-1 in figure 21.

Example 10: Chromosomal localisation

20 The 3' terminus, including the 3' UTR sequence of hVR3 was used to design two primers to amplify a product of 360 bp: sense primer 5' ATGGCCACCAGCAGGGTTAC and antisense primer 5' TCTGCCAGGTTCCAGCTG. The G3 radiation hybrid panel from Stanford University (Research Genetics, Huntsville, Alabama) was screened by PCR. The positive and negative lanes were analysed using the public web server at
25 Stanford University (<http://www-sghc.stanford.edu>). After analysis the hVR3 gene appears to be located on human chromosome 12 around markers D12S177E (lod score=15) and D12S1893 (lod score=14).

Example 11: mRNA distribution

30 The following primers (5' ACAAGAAGGCGGACATGCGG and 5' ATCTCGTGCGGTTCTCAAT) were used to obtain a PCR product from the coding region of hVR3. This amplicon was used as a probe on multi-tissue northern blots, the protocol of which is detailed in example 3, to determine the tissue distribution of the gene (figures 22A, 22B and 22C). A transcript of
35 approximately 3.8 kb was detected in the following tissues (the intensities of the

signals are indicated in brackets): trachea (very strong), kidney (strong), pancreas (strong), prostate (strong), placenta (strong), bone marrow (weak), adrenal gland (weak), lymph node (weak), spinal cord (weak), thyroid (weak), stomach (weak), lung (weak) and liver (weak).

Since these commercial blots (Clontech, Palo Alto, California, USA) should have the same amount of RNA it is interesting to note the very strong signal in the trachea lane (figure 22A). This could indicate the potential of hVR3 as a target for respiratory pathologies. It was shown by RT-PCR with the primer combination used to produce the probe that the gene is not expressed in DRG.

Example 12: Riboprobe generation for the in situ localisation of hVR3

The same probe, which was specific to hVR3 in Northern blot analysis (example 11), was used to generate a riboprobe. This hVR3 specific probe was cloned into the T7 and SP6 encoding pCRII[®]-TOPO vector (Invitrogen). This construct was then used in the *in vitro* transcription of DIG labelled RNA strands from the vectors promoters as described in the manufacturers instructions as detailed in the DIG RNA labelling kit (Roche Molecular Biochemicals). This riboprobe may be used to identify the cellular localisation of hVR3 present in tissues such as trachea, lung, pancreas, prostate, placenta and kidney.

Example 13: Mammalian Cell Expression of hVR3

Expression of hVR3 may be accomplished by transfecting a mammalian cell line such as: HEK283T, HEK293, CHO, COS, HeLa and BHK. A detailed method for both transient and stable transfection is detailed in example 6.

Example 14: Functional Analysis of hVR3

The functional analysis of hVR3 may be studied using the electrophysiology, calcium imaging and FLIPR methods as detailed in examples 7a to 7c.

Example 15: Example of a drug screen using human VR3.

A stable cell line expressing hVR3 may be used in a drug screen such as a selectivity screen using test compounds that have been identified to have an agonistic or antagonistic action on hVR1. FLIPR assay technology may be utilised to screen for hVR3 modulators as proposed in figure 15.

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Claims

1. An isolated human vanilloid receptor (hVR) protein or a variant thereof.
- 5 2. An isolated human vanilloid receptor (hVR) protein according to claim 1 which is hVR1 or a variant thereof.
3. An isolated human vanilloid receptor (hVR) protein according to claim 1
10 which is hVR3 or a variant thereof.
4. An isolated human vanilloid receptor (hVR) protein according to claim 2
having an amino acid sequence as shown in Figure 3.
5. An isolated human vanilloid receptor (hVR) protein according to claim 3
15 having an amino acid sequence as shown in Figure 18.
6. A nucleotide sequence encoding a human vanilloid receptor (hVR)
protein or a variant thereof, or a nucleotide sequence which is complementary
thereto.
20
7. A nucleotide sequence according to claim 6 encoding for an hVR1
protein or a variant thereof, or a nucleotide sequence which is complementary
thereto.
8. A nucleotide sequence according to claim 6 encoding for an hVR3
25 protein or a variant thereof, or a nucleotide sequence which is complementary
thereto.
9. A nucleotide sequence according to claim 6 which is a cDNA
30 sequence.
10. A nucleotide sequence according to claim 7 which is a cDNA sequence
11. A nucleotide sequence according to claim 8 which is a cDNA sequence

12. A nucleotide sequence according to claim 7 as shown in Figure 2.

13. A nucleotide sequence according to claim 8 as shown in Figure 17.

14. An expression vector comprising a nucleotide sequence according to any one of claims 6 to 13, which is capable of expressing an hVR protein or a variant thereof.

15. An expression vector according to claim 14 which is capable of expressing an hVR1 protein or a variant thereof.

16. An expression vector according to claim 14 which is capable of expressing an hVR3 protein or a variant thereof.

17. A stable cell line comprising an expression vector according to claim 14.

18. A stable cell line comprising an expression vector according to claim 15.

19. A stable cell line comprising an expression vector according to claim 16.

20. A stable cell line according to claim 17 which is a modified HEK293, CHO, COS, HeLa or BHK cell line.

21. A stable cell line according to claim 18 which is a modified HEK293, CHO, COS, HeLa or BHK cell line.

22. A stable cell line according to claim 19 which is a modified HEK293, CHO, COS, HeLa or BHK cell line.

23. An antibody specific for a human vanilloid receptor (hVR) protein or a variant thereof as claimed in any one of claims 1 to 5.

24. An antibody according to claim 23 which is specific for hVR1 or a variant thereof.

5 25. An antibody according to claim 23 which is specific for hVR3 or a variant thereof.

26. A method for identification of a compound which exhibits hVR
modulating activity comprising contacting a human vanilloid receptor (hVR)
10 protein or a variant thereof according to any one of claims 1 to 5 with a test
compound and detecting modulating activity or inactivity.

27. A compound which modulates hVR activity, identifiable by a method
according to claim 26.

15 28. A compound according to claim 27 for use in therapy.

29. The use of a compound according to claim 27 in the manufacture of a
medicament for treatment or prophylaxis of a disorder which is responsive to the
20 modulation of hVR activity in a human patient.

30. The use according to claim 28 wherein the disorder is pain,
neuropathic pain, inflammatory pain, chronic pain, post-operative pain,
rheumatoid arthritic pain, neuropathies, neuralgia, algesia, neurodegeneration,
25 nerve injury, stroke, ischaemia migraine, irritable bowl syndrome (IBS), a
respiratory disorder, asthma, chronic obstructive pulmonary disease (COPD), a
urological disorder, neuropathy, incontinence, interstitial cystitis or an
inflammatory disorder.

30 31. A method of treatment or prophylaxis of a disorder which is responsive
to modulation of hVR activity in a human patient which comprises administering
to said patient an effective amount of a compound according to claim 27.

35 32. A method according to claim 31 wherein the disorder is pain,
neuropathic pain, inflammatory pain, chronic pain, post-operative pain,

5 rheumatoid arthritic pain, neuropathies, neuralgia, algesia, neurodegeneration, nerve injury, stroke, ischaemia migraine, irritable bowl syndrome (IBS), a respiratory disorder, asthma, chronic obstructive pulmonary disease (COPD), a urological disorder, neuropathy, incontinence, interstitial cystitis or an inflammatory disorder.

10 33. A compound which modulates hVR activity, identifiable by a method according to claim 26, excluding the compounds capsaicin, resiniferatoxin, piperine, zingerone, polydodial, warburganal, aframodial, cinnamodial, cinnamosmolide, cinnamolide, isovelleral, scalaradial, ancistrodial, β -acaridial, scutigeral, merulidial, anandamide and capsazepine.

34. A compound according to claim 33 for use in therapy.

15 35. The use of a compound according to claim 33 in the manufacture of a medicament for treatment or prophylaxis of a disorder which is responsive to the modulation of hVR activity in a human patient.

20 36. The use according to claim 35 wherein the disorder is pain, neuropathic pain, inflammatory pain, chronic pain, post-operative pain, rheumatoid arthritic pain, neuropathies, neuralgia, algesia, neurodegeneration, nerve injury, stroke, ischaemia migraine, irritable bowl syndrome (IBS), a respiratory disorder, asthma, chronic obstructive pulmonary disease (COPD), a urological disorder, neuropathy, incontinence, interstitial cystitis or an
25 inflammatory disorder.

30 37. A method of treatment or prophylaxis of a disorder which is responsive to modulation of hVR activity in a human patient which comprises administering to said patient an effective amount of a compound according to claim 33.

35 38. A method according to claim 37 wherein the disorder is pain, neuropathic pain, inflammatory pain, chronic pain, post-operative pain, rheumatoid arthritic pain, neuropathies, neuralgia, algesia, neurodegeneration, nerve injury, stroke, ischaemia migraine, irritable bowl syndrome (IBS), a respiratory disorder, asthma, chronic obstructive pulmonary disease (COPD), a

urological disorder, neuropathy, incontinence, interstitial cystitis or an inflammatory disorder.

39. A compound identified by the method according to claim 26.

40. A compound according to claim 39 for use in therapy.

41. The use of a compound according to claim 39 in the manufacture of a medicament for treatment or prophylaxis of a disorder which is responsive to the modulation of hVR activity in a human patient.

42. The use according to claim 41 wherein the disorder is pain, neuropathic pain, inflammatory pain, chronic pain, post-operative pain, rheumatoid arthritic pain, neuropathies, neuralgia, algesia, neurodegeneration, nerve injury, stroke, ischaemia migraine, irritable bowel syndrome (IBS), a respiratory disorder, asthma, chronic obstructive pulmonary disease (COPD), a urological disorder, neuropathy, incontinence, interstitial cystitis or an inflammatory disorder.

43. A method of treatment or prophylaxis of a disorder which is responsive to modulation of hVR activity in a human patient which comprises administering to said patient an effective amount of a compound according to claim 39.

44. A method according to claim 43 wherein the disorder is pain, neuropathic pain, inflammatory pain, chronic pain, post-operative pain, rheumatoid arthritic pain, neuropathies, neuralgia, algesia, neurodegeneration, nerve injury, stroke, ischaemia migraine, irritable bowel syndrome (IBS), a respiratory disorder, asthma, chronic obstructive pulmonary disease (COPD), a urological disorder, neuropathy, incontinence, interstitial cystitis or an inflammatory disorder.

45. A method of producing an hVR protein or a variant thereof according to any one of claims 1-5 comprising introducing into an appropriate cell line a suitable vector comprising a nucleotide sequence encoding for an hVR protein or

a variant thereof, under conditions suitable for obtaining expression of the hVR protein or variant thereof.

46. A method of producing an hVR1 protein or a variant thereof comprising introducing into an appropriate cell line a suitable vector comprising a nucleotide sequence encoding for an hVR1 protein or a variant thereof, under conditions suitable for obtaining expression of the hVR1 protein or variant thereof.

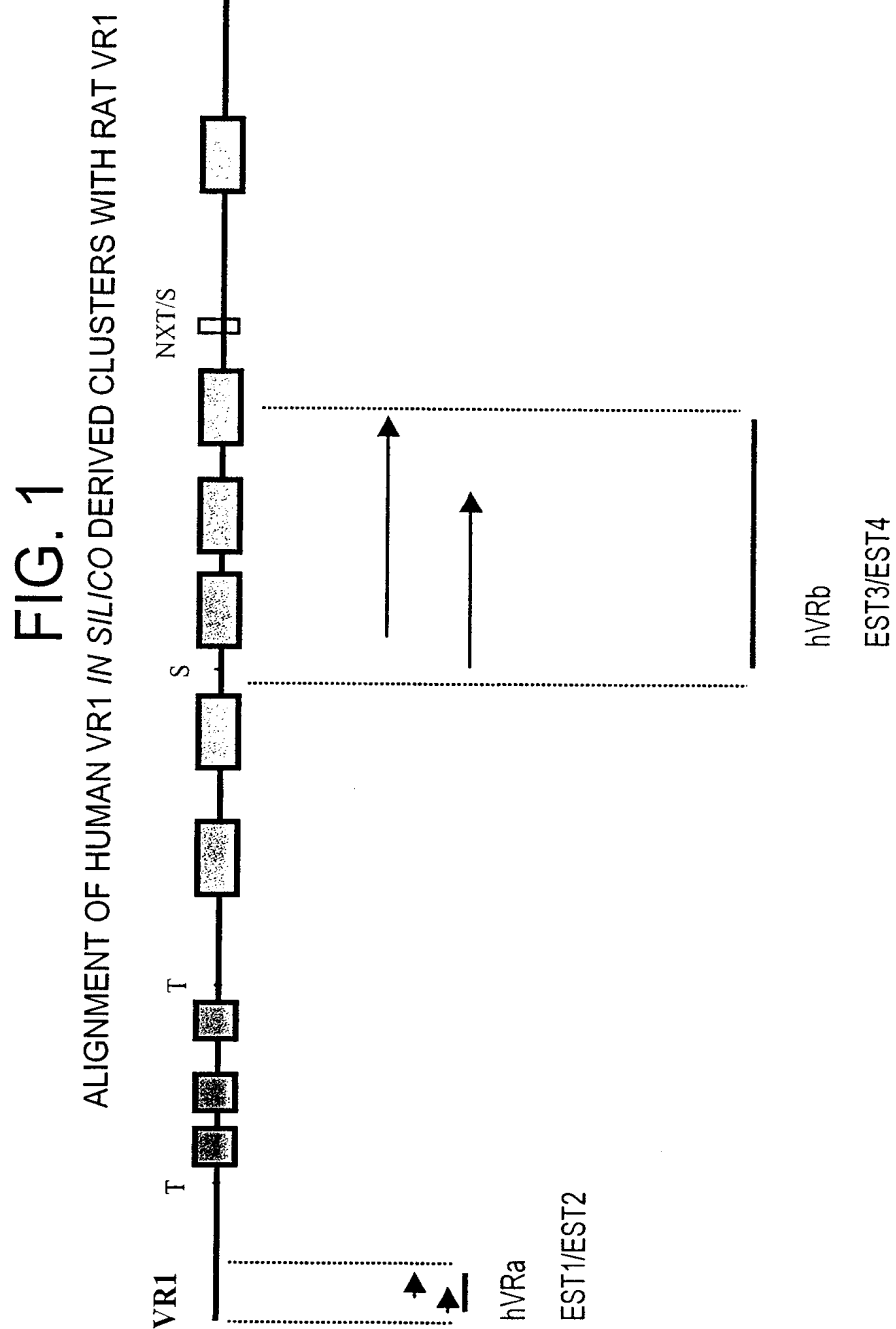
47. A method of producing an hVR3 protein or a variant thereof comprising introducing into an appropriate cell line a suitable vector comprising a nucleotide sequence encoding for an hVR3 protein or a variant thereof, under conditions suitable for obtaining expression of the hVR3 protein or variant thereof.

48. A human vanilloid receptor (hVR) protein or a variant thereof for use in a method of screening for agents useful in the treatment or prophylaxis of a disorder which is responsive to the modulation of hVR activity in a human patient

49. A human vanilloid receptor (hVR) protein according to claim 48 wherein the disorder is pain, neuropathic pain, inflammatory pain, chronic pain, post-operative pain, rheumatoid arthritic pain, neuropathies, neuralgia, algesia, neurodegeneration, nerve injury, stroke, ischaemia migraine, irritable bowel syndrome (IBS), a respiratory disorder, asthma, chronic obstructive pulmonary disease (COPD), a urological disorder, neuropathy, incontinence, interstitial cystitis or an inflammatory disorder.

50. A human vanilloid receptor (hVR) protein according to claim 48 or 49 which is hVR1 or a variant thereof.

51. A human vanilloid receptor (hVR) protein according to claim 48 or 49 which is hVR3 or a variant thereof.



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FIG. 2

hVR1 SEQUENCE INCLUDING THE 5'UTR (nt -773 TO nt 0), CODING
REGION (nt 1 TO 2517) AND 3'UTR (nt 2518 TO nt 3560)

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-773  cccccagccacacacacacacgcacacacatacacacacacacacaggttaaccattca  -714
-713  aaggccagaagcttgacagatgttgattcataaaaatgcaaagccaaaatccaaaatct  -654
-653  tgtataagctcagtggtgtggcagcgaggttgaagagcaaaggcaggccggggcacctgg  -594
-593  ctgatgatgtgtggaccggttgacagcagggcccgagtgcggtgtgggtgtgggtggg  -534
-533  ccagtctctgccgctcacctattccagggacacagtcctgcttggctcttcttgactgag  -474
-473  ccatcctcatcaccgagatcctccctgaattcagcccacgacagccacccccggccggttt  -414
-413  ccttgttctgtgtgggaagggaggcagcgcggtggttatcaacctcacctgcagaggag  -354
-353  gcacctgaggcccagagacgaggaggatgggtctaacccagaaccacagatggctctga  -294
-293  gccgggggacctgtccaccctcccaggccgacgtcagtggccgcaggactgcctgggccct  -234
-233  gctaggacctgctcacctctgaggcctctggggtgagaggttcagtcctggaaacacttca  -174
-173  gttctagggggctgggggcagcagcaagttggagttttggggtaccctgcttcacagggc  -114
-113  ccttggaaggagggcaggtggggtctaaggacaagcagtccttactttgggagtcaacc  -54
-53  ccggcgtggtggctgctgcaggttgacactgggccacagaggatccagcaaggATGAAG  6
7  AAATGGAGCAGCACAGACTTGGGGGCAGCTGCGGACCCACTCCAAAAGGACACCTGCCCA  66
67  GACCCCTGGATGGAGACCCTAACTCCAGGCCACCTCCAGCCAAGCCCCAGCTCTCCACG  126
127  GCCAAGAGCCGCACCCGGCTCTTTGGGAAGGGTGA CTGCGAGGAGGCTTTCCCGGTGGAT  186
187  TGCCCTCACGAGGAAGGTGAGCTGGACTCCTGCCCCGACCATCACAGTCAGCCCTGTTATC  246
247  ACCATCCAGAGGCCAGGAGACGGCCCCACCGGTGCCAGGCTGCTGTCCAGGACTCTGTC  306
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307	GCCGCCAGCACCGAGAAGACCCTCAGGCTCTATGATCGCAGGAGTATCTTTGAAGCCGTT	366
367	GCTCAGAATAACTGCCAGGATCTGGAGAGCCTGCTGCTCTTCCTGCAGAAGAGCAAGAAG	426
427	CACCTCACAGACAACGAGTTCAAAGACCCTGAGACAGGGAAGACCTGTCTGCTGAAAGCC	486
487	ATGCTCAACCTGCACGACGGACAGAACACCACCATCCCCCTGCTCCTGGAGATCGCGCGG	546
547	CAAACGGACAGCCTGAAGGAGCTTGTCAACGCCAGCTACACGGACAGCTACTACAAGGGC	606
607	CAGACAGCACTGCACATCGCCATCGAGAGACGCAACATGGCCCTGGTGACCCCTCCTGGTG	666
667	GAGAACGGAGCAGACGTCCAGGCTGCGGCCCATGGGGACTTCTTTAAGAAAACCAAAGGG	726
727	CGGCCTGGATTCTACTTCGGTGAAGTGGCCCTGTCCCTGGCCGCGTGACCAACCAGCTG	786
787	GGCATCGTGAAGTTCCTGCTGCAGAACTCCTGGCAGACGGCCGACATCAGCGCCAGGGAC	846
847	TCGGTGGGCAACACGGTGTGTCACGCCCTGGTGGAGGTGGCCGACAACACGGCCGACAAC	906
907	ACGAAGTTTGTGACGAGCATGTACAATGAGATTCTGATCCTGGGGGCCAAACTGCACCCG	966
967	ACGCTGAAGCTGGAGGAGCTCACCAACAAGAAGGGAATGACGCCGCTGGCTCTGGCAGCT	1026
1027	GGGACCGGGAAGATCGGGGTCTTGGCCTATATTCTCCAGCGGGAGATCCAGGAGCCCGAG	1086
1087	TGCAGGCACCTGTCCAGGAAGTTCACCGAGTGGGCCTACGGGCCCGTGCACTCCTCGCTG	1146
1147	TACGACCTGTCTCTGCATCGACACCTGCGAGAAGAACTCGGTGCTGGAGGTGATCGCCTAC	1206
1207	AGCAGCAGCGAGACCCCTAATCGCCACGACATGCTCTTGGTGGAGCCGCTGAACCGACTC	1266
1267	CTGCAGGACAAGTGGGACAGATTTCGTCAAGCGCATCTTCTACTTCAACTTCCTGGTCTAC	1326
1327	TGCCTGTACATGATCATCTTCACCATGGCTGCCTACTACAGGCCCGTGGATGGCTTGCCT	1386
1387	CCCTTTAAGATGGAAAAAATTGGAGACTATTTCCGAGTTACTGGAGAGATCCTGTCTGTG	1446

FIG. 2_{CONT'D}

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1447 TTAGGAGGAGTCTACTTCTTTTTCCGAGGGATTTCAGTATTTCTGCAGAGGCGGCCGTCG 1506

1507 ATGAAGACCCCTGTTTGTGGACAGCTACAGTGAGATGCTTTTCTTTCTGCAGTCACTGTTC 1566

1567 ATGCTGGCCACCGTGGTGTCTTCTCAGCCACCTCAAGGAGTATGTGGCTTCCATGGTA 1626

1627 TTCTCCCTGGCCTTGGGCTGGACCAACATGCTCTACTACACCCGCGGTTTCCAGCAGATG 1686

1687 GGCATCTATGCCGTCATGATAGAGAAGATGATCCTGAGAGACCTGTGCCGTTTCATGTTT 1746

1747 GTCTACATCGTCTTCTTGTTCGGGTTTTCCACAGCGGTGGTGACGCTGATTGAAGACGGG 1806

1807 AAGAATGACTCCCTGCCGTCTGAGTCCACGTGCGCACAGGTGGCGGGGGCCTGCCTGCAGG 1866

1867 CCCCCGATAGCTCCTACAACAGCCTGTACTCCACCTGCCTGGAGCTGTTCAAGTTCACC 1926

1927 ATCGGCATGGGCGACCTGGAGTTCACTGAGAACTATGACTTCAAGGCTGTCTTCATCATC 1986

1987 CTGCTGCTGGCCTATGTAATTCTCACCTACATCCTCCTGCTCAACATGCTCATCGCCCTC 2046

2047 ATGGGTGAGACTGTCAACAAGATCGCACAGGAGAGCAAGAACATCTGGAAGCTGCAGAGA 2106

2107 GCCATCACCATCCTGGACACGGAGAAGAGCTTCCTTAAGTGCATGAGGAAGGCCTTCCGC 2166

2167 TCAGGCAAGCTGCTGCAGGTGGGGTACACACCTGATGGCAAGGACGACTACCGGTGGTGC 2226

2227 TTCAGGGTGGACGAGGTGAACTGGACCACCTGGAACACCAACGTGGGCATCATCAACGAA 2286

2287 GACCCGGGCAACTGTGAGGGCGTCAAGCGCACCCCTGAGCTTCTCCCTGCGGTCAAGCAGA 2346

2347 GTTTCAGGCAGACACTGGAAGAACTTTGCCCTGGTCCCCCTTTTAAGAGAGGCAAGTGCT 2406

2407 CGAGATAGGCAGTCTGCTCAGCCCGAGGAAGTTTATCTGCGACAGTTTTCAGGGTCTCTG 2466

2467 AAGCCAGAGGACGCTGAGGTCTTCAAGAGTCCTGCCGCTTCCGGGGAGAAGtgaggacgt 2526

2527 cacgcagacagcactgtcaacactgggccttaggagacccccgttgccacggggggctgct 2586

FIG.2CONT'D

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2587 gagggaaacaccagtgtctgtcagcagcctggcctgggtctgtgcctgccagcatgttcc 2646
2647 caaatctgtgctggacaagctgtgggaagcggttcttggaagcatggggagtgatgtacat 2706
2707 ccaaccgctcactgtccccaagtgaatctcctaacagactttcagggtttttactcacttta 2766
2767 ctaaacagtttggtggtcagtcctctactgggacatgttaggcccttgttttctttgatt 2826
2827 ttattcttttctgtgagacagagttcactcttggtgccaggctggagtgcagtgggtgtg 2886
2887 atcttgggtcactgcaacctctgctcccggttcaagcgattcttctgcttcagtctccc 2946
2947 aagtagcttggattacaggtgagcactaccacgcccggctaatttttgtatttttaatag 3006
3007 agacgggggtttcaccatgttggccaggctggtctcgaactcttgacctcaggtgatctgc 3066
3067 ccgccttggcctcccaaagtgtgggattacaggtgtgagccgctgcgctcggccttctt 3126
3127 tgattttatattattaggagcaaaagtaaataagcccaggaaaacacctttgggaacaa 3186
3187 actcttcctttgatggaaaatgcagaggcccttctctctgtgccgtgcttgctcctctt 3246
3247 acctgcccgggtgggtttgggggtgttggtgtttcctccctggagaagatgggggaggctg 3306
3307 tcccactcccagctctggcagaatcaagctgttgagcagtgcccttcttcacatccttcctt 3366
3367 acgatcaatcacagtctccagaagatcagctcaattgctgtgcagggttaaaactacagaa 3426
3427 ccacatcccaaaggtacctggtaagaatgtttgaaagatcttccattttctaggaacccca 3486
3487 gtctgtcttctccgcaatggcacatgcttccactccatccatactggcatcctcaaataa 3546
3547 acagatatgtatacaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaa 3591

FIG. 2_{CONT'D}

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FIG. 3

NUCLEOTIDE AND AMINO ACID SEQUENCE OF hVR1 INCLUDING
THE 5'UTR (nt -773 TO nt 0), CODING REGION (nt TO 2517) AND
3'UTR (nt 2518 TO nt 3560)

-773	ccccagccacacacacacacgcacacacatacacacacacacacaggcttaaccattca	-714
-713	aaggccagaagcttgacagatggttgattcataaaaatgcaaaagccaaaatccaaaatct	-654
-653	tgtataagctcagtggtgtggcagcgaggttgaagagcaaaggcaggccgggcacctgg	-594
-593	ctgatgatgtgtggacccgttgacacagcagggcccgagtgcggtgtgggtgtgggtggg	-534
-533	ccagtctctgccgctcacccattccagggacacagtcctgcttggtctctctggactgag	-474
-473	ccatcctcatcacccgagatcctccctgaattcagcccacgacagccaccccgccgctttt	-414
-413	ccttggtctgtgtgggaagggaggcagcgcggtggttatcaacctcaccctgcagaggag	-354
-353	gcacctgagggccagagacgaggaggatgggtctaaccacagaaccacagatggctctga	-294
-293	gccgggggacctgtccaccctcccaggccgacgtcagtgggccgaggactgcctgggacct	-234
-233	gctaggacctgctcacctctgaggacctctggggtgagaggttcagtcctggaaacacttca	-174
-173	gttctagggggctgggggcagcagcaagttggagttttggggtaccctgcttcacagggc	-114
-113	ccttggaaggaggggcaggtggggtctaaggacaagcagtccttactttgggagtcaacc	-54
-53	ccggcggtgggtgctgctgcaggttgcacactggggccacagaggatccagcaaggATGAAG	6
1		M K 2
7	AAATGGAGCAGCACAGACTTGGGGGCAGCTGCGGACCCACTCCAAAAGGACACCTGCCCA	66
3	K W S S T D L G A A A D P L Q K D T C P	22
67	GACCCCTGGATGGAGACCCCTAACTCCAGGCCACCTCCAGCCAAGCCCCAGCTCTCCACG	126
23	D P L D G D P N S R P P P A K P Q L S T	42
127	GCCAAGAGCCGCACCCGGCTCTTTGGGAAGGGTGACTCGGAGGAGGCTTTCCCGGTGGAT	186
43	A K S R T R L F G K G D S E E A F P V D	62
187	TGCCCTCACGAGGAAGGTGAGCTGGACTCCTGCCCGACCATCACAGTCAGCCCTGTTATC	246
63	C P H E E G E L D S C P T I T V S P V I	82
247	ACCATCCAGAGGCCAGGAGACGGCCCCACCGGTGCCAGGCTGCTGTCCCAGGACTCTGTC	306
83	T I Q R P G D G P T G A R L L S Q D S V	102
307	GCCGCCAGCACCGAGAAGACCCTCAGGCTCTATGATCGCAGGAGTATCTTTGAAGCCGTT	366
103	A A S T E K T L R L Y D R R S I F E A V	122
367	GCTCAGAATAACTGCCAGGATCTGGAGAGCCTGCTGCTCTTCCTGCAGAAGAGCAAGAAG	426
123	A Q N N C Q D L E S L L L F L Q K S K K	142
427	CACCTCACAGACAACGAGTTCAAAGACCCTGAGACAGGGAAGACCTGTCTGCTGAAAGCC	486
143	H L T D N E F K D P E T G K T C L L K A	162
487	ATGCTCAACCTGCACGACGGACAGAACACCACCATCCCCCTGCTCCTGGAGATCGCGCGG	546

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163	M L N L H D G Q N T T I P L L L E I A R	182
547	CAAACGGACAGCCTGAAGGAGCTTGTCAACGCCAGCTACACGGACAGCTACTACAAGGGC	606
183	Q T D S L K E L V N A S Y T D S Y Y K G	202
607	CAGACAGCACTGCACATCGCCATCGAGAGACGCAACATGGCCCTGGTGACCCTCCTGGTG	666
203	Q T A L H I A I E R R N M A L V T L L V	222
667	GAGAACGGAGCAGACGTCCAGGCTGCGGCCCATGGGGACTTCTTTAAGAAAACCAAAGGG	726
223	E N G A D V Q A A A H G D F F K K T K G	242
727	CGGCCTGGATTCTACTTCCGGTGAAGTGGCCCTGTCCCTGGCCGCGTGACCAACCAGCTG	786
243	R P G F Y F G E L P L S L A A C T N Q L	262
787	GGCATCGTGAAGTTCCTGCTGCAGAACTCCTGGCAGACGGCCGACATCAGCGCCAGGGAC	846
263	G I V K F L L Q N S W Q T A D I S A R D	282
847	TCGGTGGGCAACACGGTGCTGCACGCCCTGGTGGAGGTGGCCGACAACACGGCCGACAAC	906
283	S V G N T V L H A L V E V A D N T A D N	302
907	ACGAAGTTTGTGACGAGCATGTACAATGAGATTCTGATCCTGGGGGCCAAACTGCACCCG	966
303	T K F V T S M Y N E I L I L G A K L H P	322
967	ACGCTGAAGCTGGAGGAGCTCACCAACAAGAAGGGAATGACGCCGCTGGCTCTGGCAGCT	1026
323	T L K L E E L T N K K G M T P L A L A A	342
1027	GGGACCGGGAAGATCGGGGTCTTGGCCTATATTCTCCAGCGGGAGATCCAGGAGCCCGAG	1086
343	G T G K I G V L A Y I L Q R E I Q E P E	362
1087	TGCAGGCACCTGTCCAGGAAGTTCACCGAGTGGGCCTACGGGCCCGTGCACTCCTCGCTG	1146
363	C R H L S R K F T E W A Y G P V H S S L	382
1147	TACGACCTGTCTGTCATCGACACCTGCGAGAAGAACTCGGTGCTGGAGGTGATCGCCTAC	1206
383	Y D L S C I D T C E K N S V L E V I A Y	402
1207	AGCAGCAGCGAGACCCCTAATCGCCACGACATGCTCTTGGTGGAGCCGCTGAACCGACTC	1266
403	S S S E T P N R H D M L L V E P L N R L	422
1267	CTGCAGGACAAGTGGGACAGATTTCGTCAAGCGCATCTTCTACTTCAACTTCCTGGTCTAC	1326
423	L Q D K W D R F V K R I F Y F N F L V Y	442
1327	TGCCTGTACATGATCATCTTCACCATGGCTGCCTACTACAGGCCGCTGGATGGCTTGCCT	1386
443	C L Y M I I F T M A A Y Y R P V D G L P	462
1387	CCCTTTAAGATGGAAAAAATTGGGAGACTATTTCCGAGTTACTGGAGAGATCCTGTCTGTG	1446
463	P F K M E K I G D Y F R V T G E I L S V	482
1447	TTAGGAGGAGTCTACTTCTTTTTCCGAGGGATTTCAGTATTTCTGCAGAGGCGGCCGTCG	1506
483	L G G V Y F F F R G I Q Y F L Q R R P S	502
1507	ATGAAGACCCTGTTTGTGGACAGCTACAGTGAGATGCTTTTCTTCTGCAGTCACTGTTC	1566
503	M K T L F V D S Y S E M L F F L Q S L F	522
1567	ATGCTGGCCACCGTGGTGCTGTACTTCAGCCACCTCAAGGAGTATGTGGCTTCCATGGTA	1626
523	M L A T V V L Y F S H L K E Y V A S M V	542
1627	TTCTCCCTGGCCTTGGGCTGGACCAACATGCTCTACTACACCCGCGGTTTCCAGCAGATG	1686

FIG. 3_{CONT'D}

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543 F S L A L G W T N M L Y Y T R G F Q Q M 562

1687 GGCATCTATGCCGTCATGATAGAGAAGATGATCCTGAGAGACCTGTGCCGTTTCATGTTT 1746
563 G I Y A V M I E K M I L R D L C R F M F 582

1747 GTCTACATCGTCTTCTTGTTCGGGTTTTCCACAGCGGTGGTGACGCTGATTGAAGACGGG 1806
583 V Y I V F L F G F S T A V V T L I E D G 602

1807 AAGAATGACTCCCTGCCGTCTGAGTCCACGTGCGACAGGTGGCGGGGGCCTGCCTGCAGG 1866
603 K N D S L P S E S T S H R W R G P A C R 622

1867 CCCCCGATAGCTCCTACAACAGCCTGTACTCCACCTGCCTGGAGCTGTTCAAGTTCACC 1926
623 P P D S S Y N S L Y S T C L E L F K F T 642

1927 ATCGGCATGGGCGACCTGGAGTTCACTGAGAATATGACTTCAAGGCTGTCTTCATCATC 1986
643 I G M G D L E F T E N Y D F K A V F I I 662

1987 CTGCTGCTGGCCTATGTAATTCTCACCTACATCCTCCTGCTCAACATGCTCATCGCCCTC 2046
663 L L L A Y V I L T Y I L L L N M L I A L 682

2047 ATGGGTGAGACTGTCAACAAGATCGCACAGGAGAGCAAGAACATCTGGAAGCTGCAGAGA 2106
683 M G E T V N K I A Q E S K N I W K L Q R 702

2107 GCCATCACCATCCTGGACACGGAGAAGAGCTTCTTAAGTGCATGAGGAAGGCCTTCCGC 2166
703 A I T I L D T E K S F L K C M R K A F R 722

2167 TCAGGCAAGCTGCTGCAGGTGGGGTACACACCTGATGGCAAGGACGACTACCGGTGGTGC 2226
723 S G K L L Q V G Y T P D G K D D Y R W C 742

2227 TTCAGGGTGGACGAGGTGAACTGGACCACCTGGAACACCAACGTGGGCATCATCAACGAA 2286
743 F R V D E V N W T T W N T N V G I I N E 762

2287 GACCCGGGCAACTGTGAGGGCGTCAAGCGCACCTGAGCTTCTCCCTGCGGTCAAGCAGA 2346
763 D P G N C E G V K R T L S F S L R S S R 782

2347 GTTTCAGGCAGACACTGGAAGAACTTTGCCCTGGTCCCCCTTTTAAGAGAGGCAAGTGCT 2406
783 V S G R H W K N F A L V P L L R E A S A 802

2407 CGAGATAGGCAGTCTGCTCAGCCCGAGGAAGTTTATCTGCGACAGTTTTCAGGGTCTCTG 2466
803 R D R Q S A Q P E E V Y L R Q F S G S L 822

2467 AAGCCAGAGGACGCTGAGGTCTTCAAGAGTCCTGCCGCTTCCGGGGAGAAGtgaggacgt 2526
823 K P E D A E V F K S P A A S G E K 839

2527 cacgcagacagcactgtcaacactgggccttaggagaccccgttgccacggggggctgct 2586

2587 gagggaacaccagtgtctctgtcagcagcctggcctggtctgtgcctgcccagcatgttcc 2646

2647 caaatctgtgctggacaagctgtgggaagcgttcttggaagcatggggagtgtgtacat 2706

2707 ccaaccgtcactgtccccaagtgaatctcctaacagacttttcagggtttttactcacttta 2766

2767 ctaaacagtttggtatgggtcagtcctctactgggacatgttaggcccttggtttcttttgatt 2826

2827 ttattcttttctgtgagacagagttcactcttgttgcccaggctggagtgacgtggtgtg 2886

2887 atcttggtcactgcaacctctgctcccgggttcaagcgattcttctgcttcagtcctccc 2946

FIG. 3CONT'D

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2947	aagtagcttggattacaggtgagcactaccacgcccggctaatttttgtatttttaatag	3006
3007	agacgggggtttcaccatggtggccaggctggtctogaactcttgacctcaggtgatctgc	3066
3067	ccgccttggcctcccaaagtgcctgggattacaggtgtgagccgctgcgctcggccttctt	3126
3127	tgattttatattattaggagcaaaagtaaatgaagcccaggaaaacacctttggaacaa	3186
3187	actcttcctttgatggaaaatgcagaggcccttcctctctgtgcgctgcttgctcctctt	3246
3247	acctgcccgggtgggtttgggggtgttggtgtttcctccctggagaagatgggggaggctg	3306
3307	tcccactcccagctctggcagaatcaagctggtgcagcagtgcccttcttcacctcctt	3366
3367	acgatcaatcacagtctccagaagatcagctcaattgctgtgcagggttaaaactacagaa	3426
3427	ccacatcccaaagggtacctggtgaagaatgtttgaaagatcttccatttctaggaacccca	3486
3487	gtcctgcttctccgcaatggcacatgcttccactccatccatactggcatcctcaaataa	3546
3547	acagatatgtatacaaaaaaaaaaaaaaaaaaaaaaaaaaaaaa	3591

FIG. 3_{CONT'D}

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FIG. 4


AMINO ACID SEQUENCE OF hVR1

1 MKKWSSTD LG AAADPLQKDT CPDPLD GPN SRPPPAKPQL STAKSRTLRF
 51 GKGDSEEA FP VDCPHEEGEL DSCPTITVSP VITIQRPGDG PTGARLLSQD
 101 SVAASTEKTL RLYDRRSIFE AVAQNNCQDL ESLLLFLQKS KKHLTDNEFK
 151 DPETGKTCLL KAMLNLDHGQ NTTIPLLEI ARQTD SLKEL VNASYTDSYY
 201 KGQTALHIAI ERNMALVTL LVENGADVQA AAHGDFFKKT KGRPGFYFGE
 251 LPLSLAACTN QLGIVKFLQ NSWQTADISA RDSVGNTVLH ALVEVADNTA
 301 DNTKFVTS MY NEILILGAKL HPTL KLEELT NKKGMTPLAL AAGTGKIGVL
 351 AYILQREIQE PECHLSRKF TEWAYGPVHS SLYDLSCIDT CEKNSVLEVI
 401 AYSSSETPNR HDMLLVEPLN RLLQDKWDRF VKRIFVFNFL VYCIYMIET
 451 MAAYRPVDG LPPFKMEKIG DYFRVTGEIL SVLCGVYFFF RGIQYFLQRR
 501 PSMKTLFVI SYSEMTEFELQS LEMLATVVLYES HLKEYVAS MVESLALGWT
 551 NMELYTRGFO QMGIVAVMIE KMILRI LCREMEVYIVFLEG FSTAVVTLIE
 601 DGKNDSLPSE STSHRWGP A CRPPDSSYNS LYSTCLELFK FTIGMGDLEF
 651 TENYDEKAVF LILLHAYVILF VLYLHHENMITE ALMGETVNKI AQESKNIWKL
 701 QRAITILDTE KSFLKCMRKA FRSGKLLQVG YTPDGKDDYR WCFRVDEVNW
 751 TTWNTNVGII NEDPGNCXGV KRTLSFSLRS SRVSGRHWKN FALVPLLREA
 801 SARDRQSAQP EEVYLRQFSG SLKPDAEVF KSPAASGEK*

Key

T/S predicted phosphorylation sites

 Transmembrane domains

 Ankyrin binding domains

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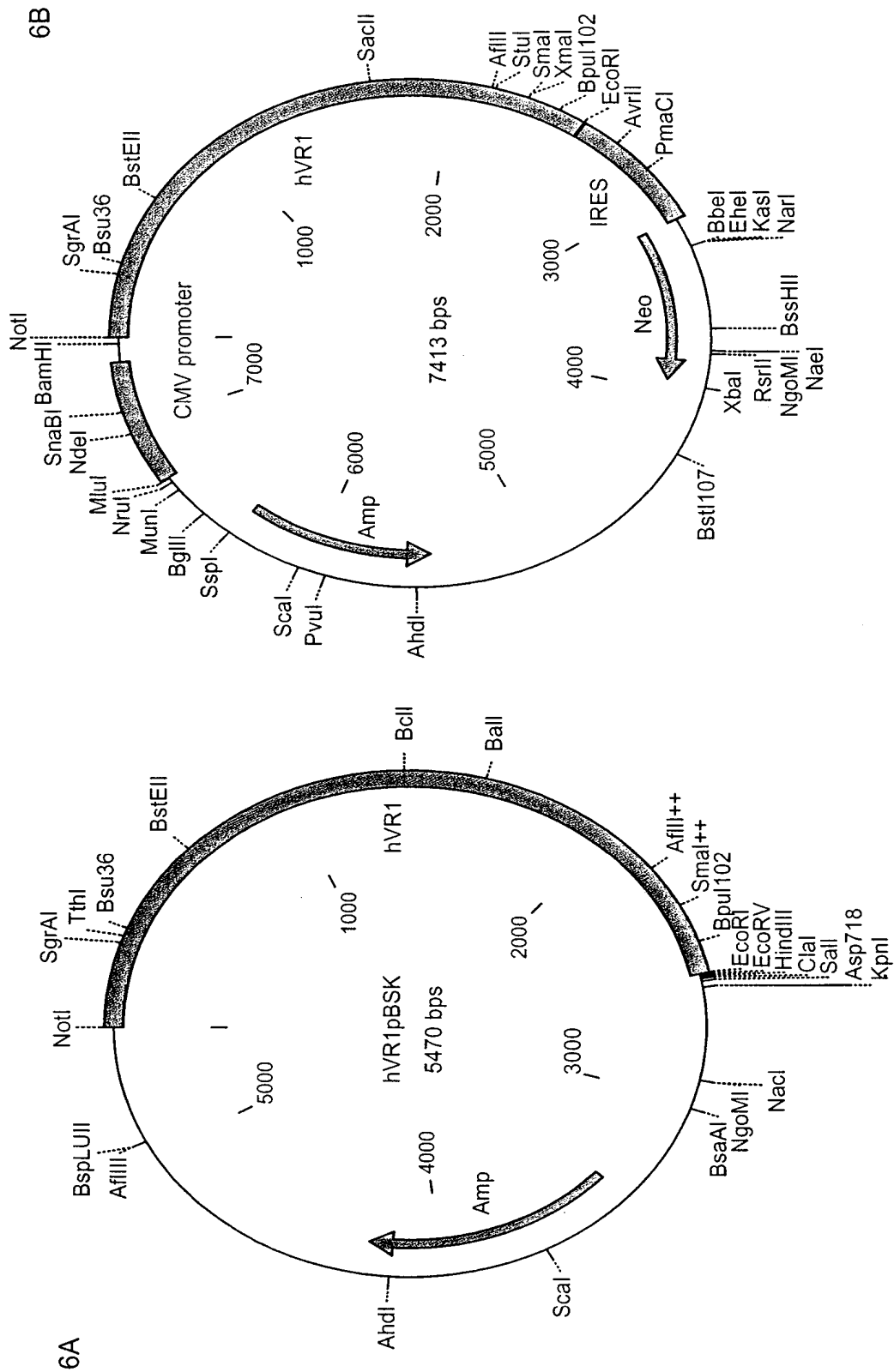
FIG. 5

COMPARISON OF THE AMINO ACID SEQUENCE OF THE RAT (VR1)
AND HUMAN (hVR1) VANILLOID PROTEINS.

	10	20	30	40	50
VR1	MEQRASLDSEES	ESPPQENSCLD	PPDRDPNCKPP	VPKPHIFTTR	SRTRLF
hVR1	MKKWSSTD	LGAADPLQKDT	CPDPLDGPNS	RPPPAKPQLS	TAKSRTRLF
	60	70	80	90	100
VR1	GKGDSEEA	SPLD	CPYEEGLAS	CPITVSSVLT	IQRPDGPAS
hVR1	GKGDSEEA	FVDCPHEE	GELDS	CPITVSPVIT	IQRPDGP
	110	120	130	140	150
VR1	SVSAG.EKPP	RLYDRRSIF	DAVAQSN	CQELSLP	FLQRSKKRL
hVR1	SVAASTE	KTLRLYDRRS	IFEAVAQ	NNCQDLES	LLFLQSKKHL
	160	170	180	190	200
VR1	DPETGKT	CLLKAM	NLHNGOND	TIALLLD	VARKTDSL
hVR1	DPETGKT	CLLKAM	NLHDGQNT	TIPLLLE	IARQD
	210	220	230	240	250
VR1	KGQTALH	IAIERN	NMTLVTL	LVEN	GADVQAA
hVR1	KGQTALH	IAIERN	NMALVT	LLVEN	GADVQAA
	260	270	280	290	300
VR1	LPLSLA	ACTNQL	AIVKFL	LQNSWQ	PADISAR
hVR1	LPLSLA	ACTNQL	GIVKFL	LQNSWQ	TADISAR
	310	320	330	340	350
VR1	DNTKFV	TSMYNE	ILILGA	KLHPTL	KLEEITN
hVR1	DNTKFV	TSMYNE	ILILGA	KLHPTL	KLEELTN
	360	370	380	390	400
VR1	AYILQRE	IHEPECR	HL	SRKFTE	WAYG
hVR1	AYILQRE	IHEPECR	HL	SRKFTE	WAYG
	410	420	430	440	450
VR1	AYSSSE	TPNRHD	MLLVE	PLNRL	LQDKWDR
hVR1	AYSSSE	TPNRHD	MLLVE	PLNRL	LQDKWDR
	460	470	480	490	500
VR1	AAAYYR	PVEGLP	PKLKW	TVGDFR	VTGEIL
hVR1	MAAYYR	PVDGLP	PKMEK	.IGDYF	RVVTGEIL
	510	520	530	540	550
VR1	RPSLK	SFVDSY	SEILFF	VQSLF	MVSVVLY
hVR1	RPSMK	TFVDSY	SEMLFF	LQSLF	MVSVVLY
	560	570	580	590	600
VR1	TNMLYY	TRGFQ	QMG	IYAV	MIEMIL
hVR1	TNMLYY	TRGFQ	QMG	IYAV	MIEMIL
	610	620	630	640	650
VR1	EDGKNN	SLPMES	TPHKCR	GSACK.	PGNSYN
hVR1	EDGKND	SLPSES	TSHRWR	GPACRP	PDSSYN
	660	670	680	690	700
VR1	FTENYD	FKAVFI	IILLAY	VILTYI	ILLNML
hVR1	FTENYD	FKAVFI	IILLAY	VILTYI	ILLNML
	710	720	730	740	750
VR1	LQRAIT	ILDTEK	SFLKCM	RKA	FRSGKLL
hVR1	LQRAIT	ILDTEK	SFLKCM	RKA	FRSGKLL
	760	770	780	790	800
VR1	WTTWNT	NVGI	INED	PGNCE	GVKRTL
hVR1	WTTWNT	NVGI	INED	PGNCE	GVKRTL
	810	820	830		
VR1	ASTRDR	HATQ	QEEV	OLKHYT	GS
hVR1	ASARDR	QSAQ	PEEV	YLRQ	FS

FIG. 6

FULL-LENGTH hRV1 CLONED INTO (A) pBLUESCRIPT SK(+) (hVR1pBSK) AND (B) pCIN5-NEW (hVR1pCIN5) VIA NotI/EcoRI RESTRICTION SITES.



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FIG. 7

SLOT HYBRIDISATION WITH hVR1 PROBE

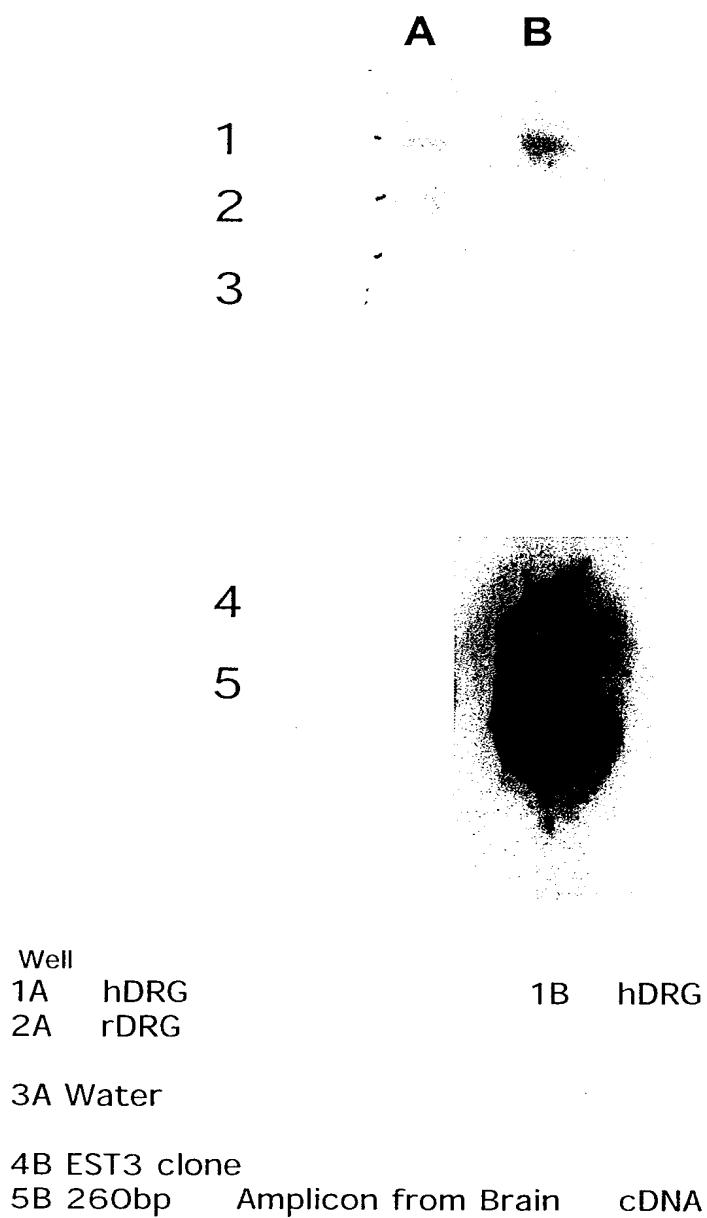


FIG. 8

WESTERN BLOT PROBED WITH ANTI-hVR1 ANTIBODIES.
ARROW POINTS TO hVR1 SPECIFIC BAND

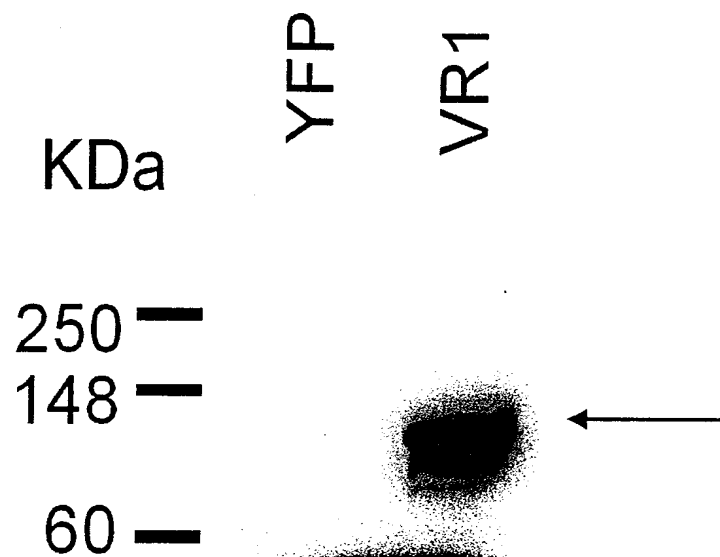
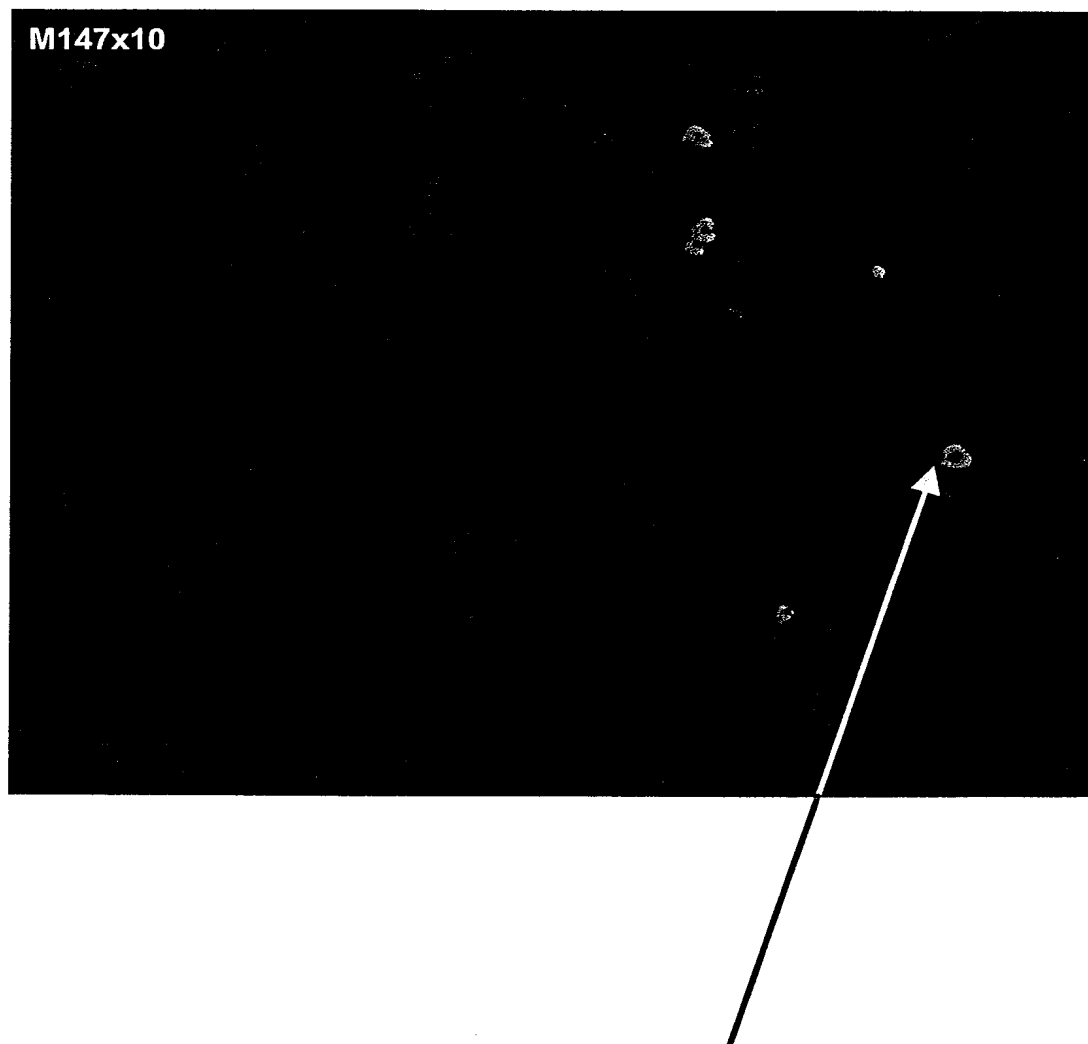


FIG. 9

IN SITU LOCALISATION OF VR1 IN RAT DRG TISSUE SECTIONS.
ARROW POINTS TO A VR1 EXPRESSING SMALL DIAMETER
($<25\mu\text{m}$) NEURONE CELL BODY, MAGNIFICATION USED 147×10 .



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FIG. 10A

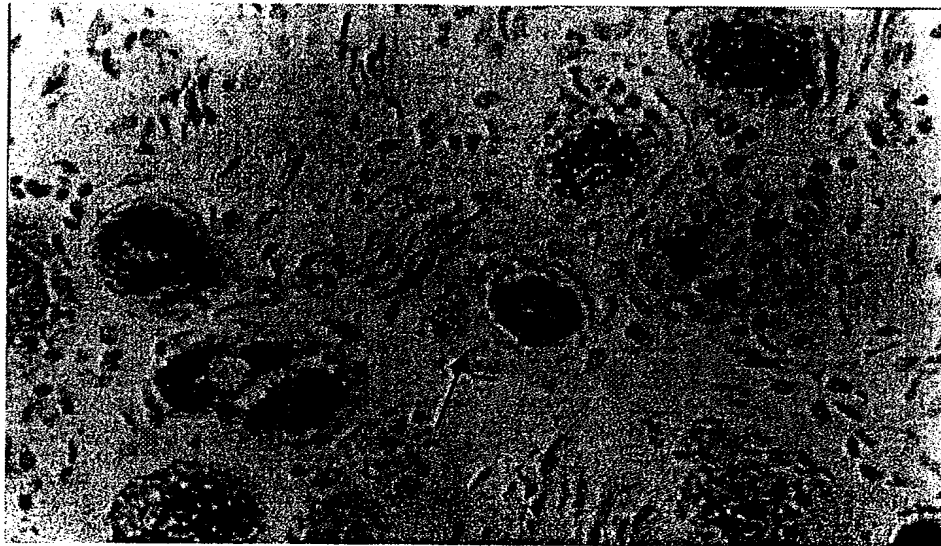


FIG. 10B

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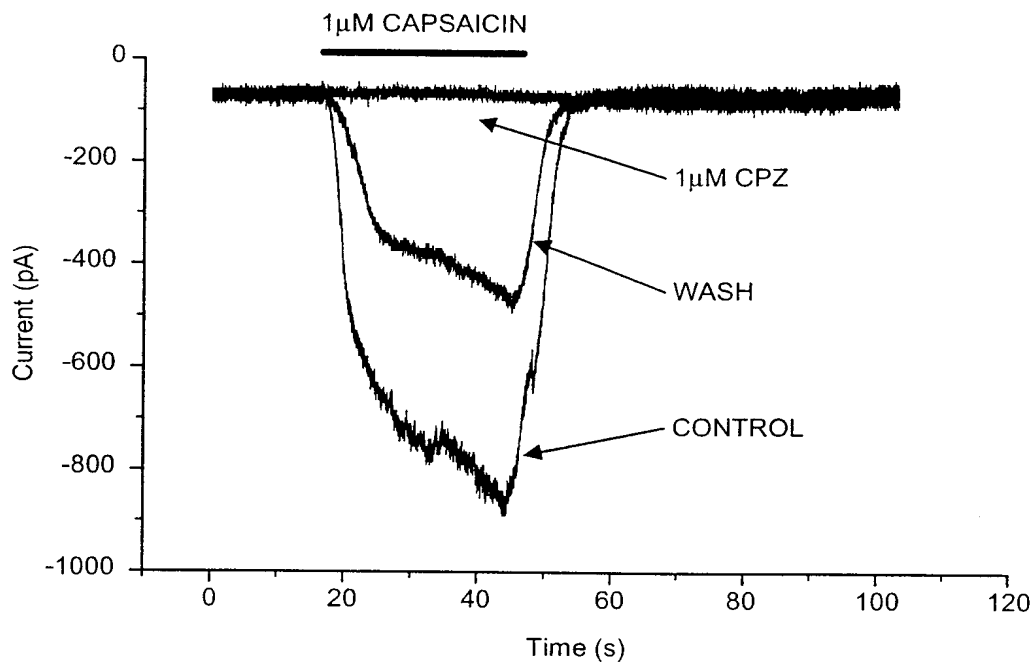
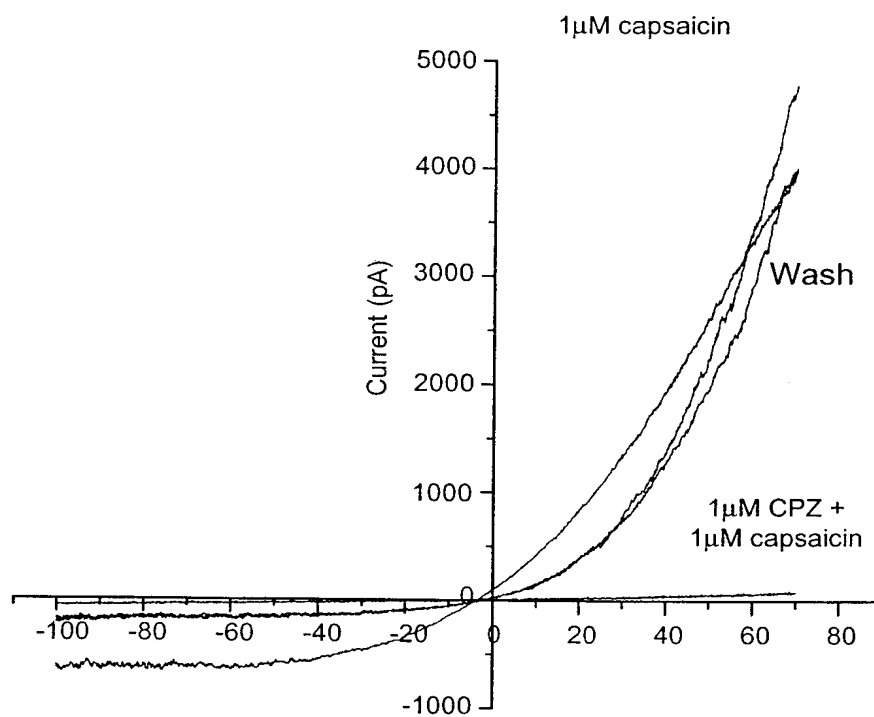


FIG. 11A



SOLUTIONS
OUTSIDE 140mM Na⁺ 2mM Ca²⁺
INSIDE 140mM Cs⁺

FIG. 11B

FIG. 12A

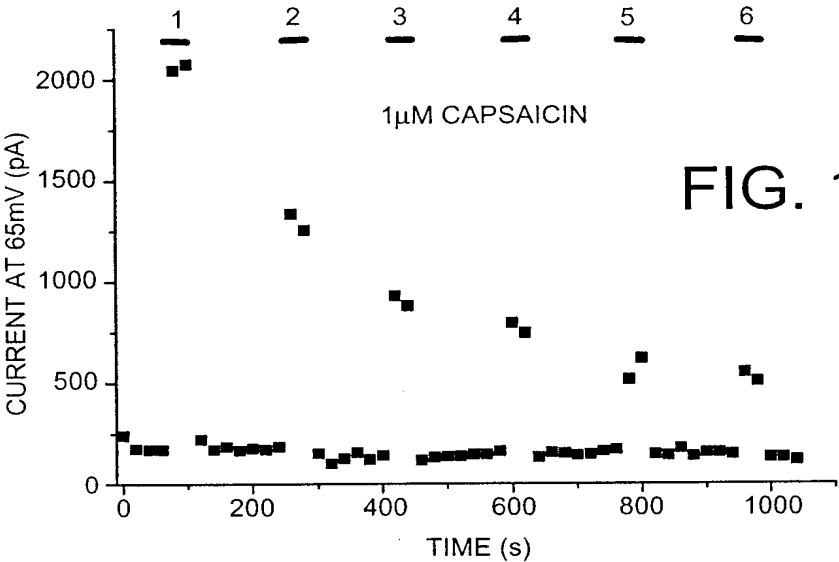
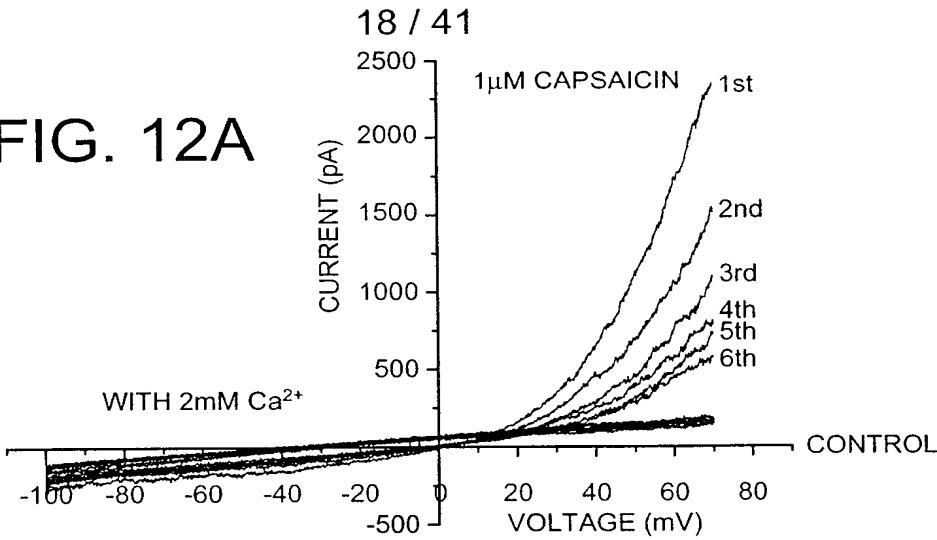


FIG. 12B

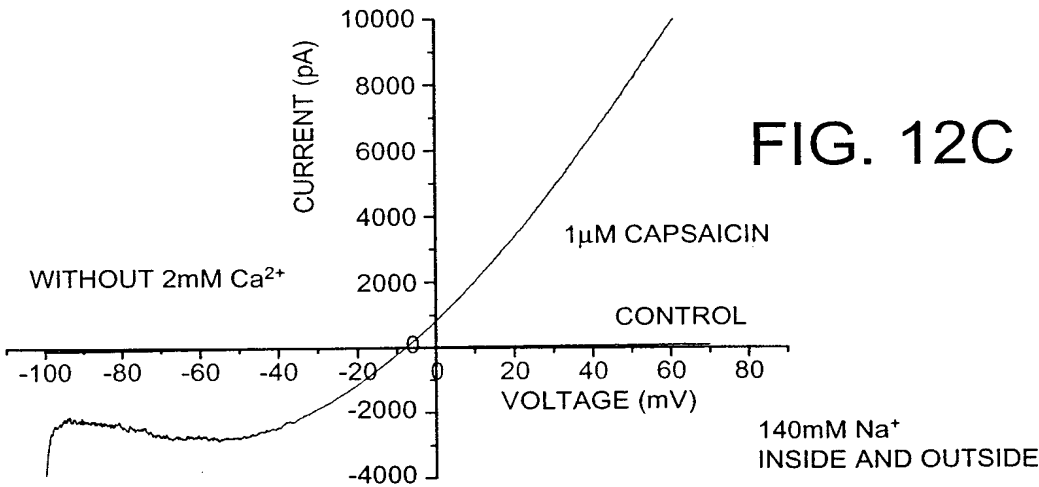
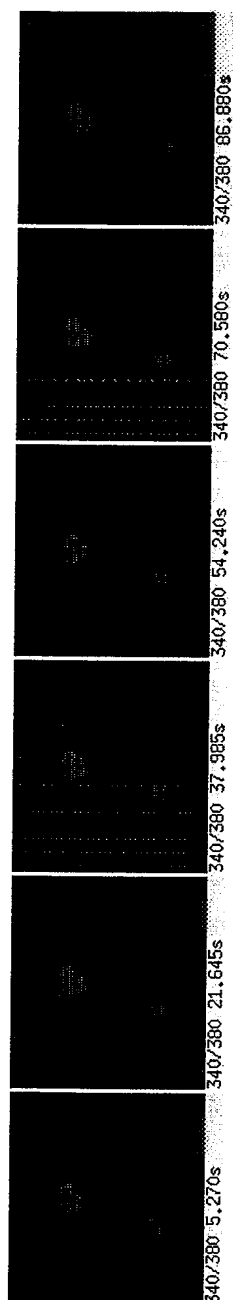


FIG. 12C

13A pCIN5-new in HEK293T, 24hr transient expression, stimulated with 3 μ M capsaicin at time point 52 secs of time course



13B hVR1pCIN5 in HEK293T, 24hr expression, stimulated with 1 μ M capsaicin at time point 52 seconds



13C hVR1pCIN5 in HEK293T, 24hr transient expression, 20 min pre-incubation with 10 μ M capsazepine, stimulated with 1 μ M capsaicin at time point 52 seconds of time course

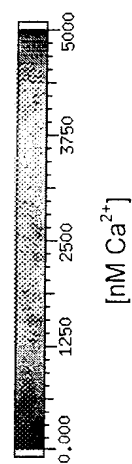
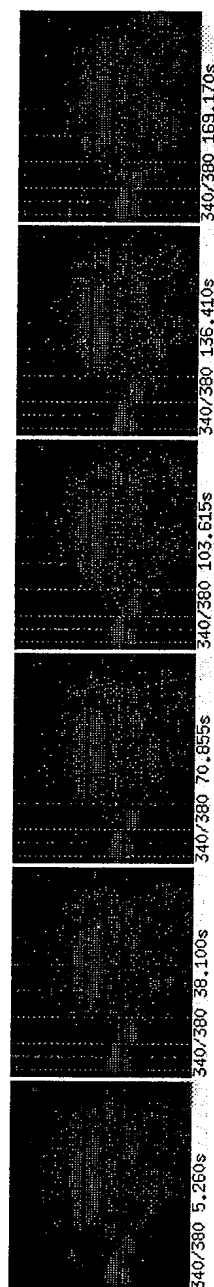
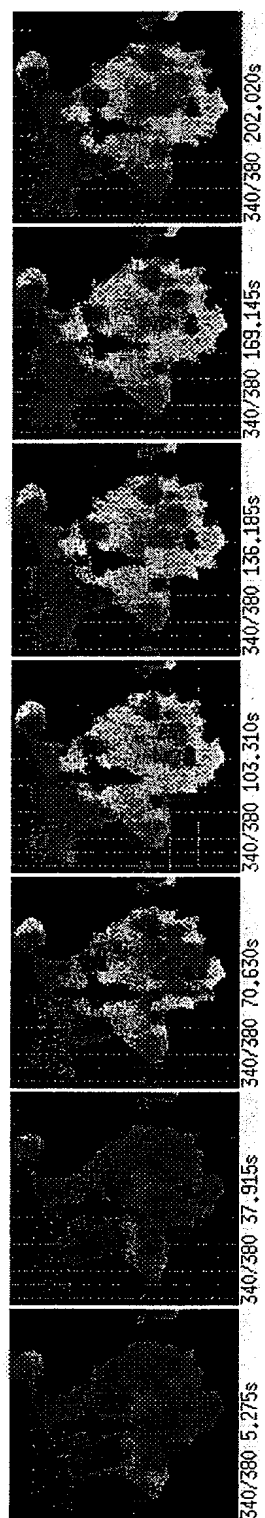


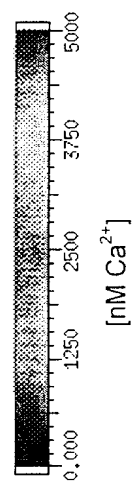
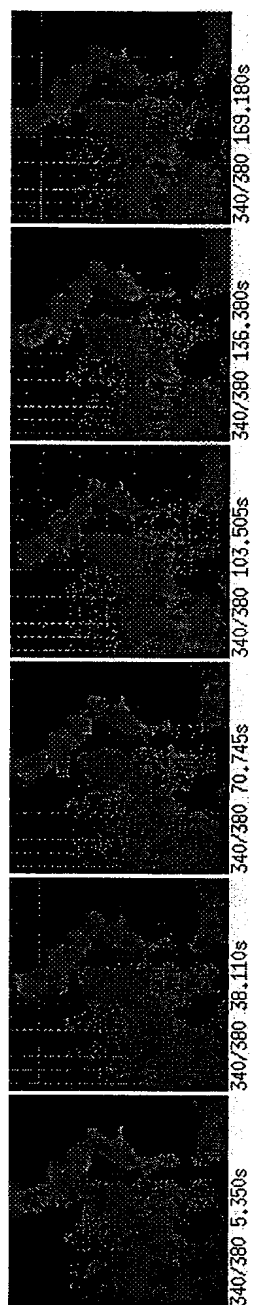
FIG. 13

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13D hVR1pCIN5 in HEK293T, 24hr transient expression, stimulated with 10uM anandamide at time point 52 seconds

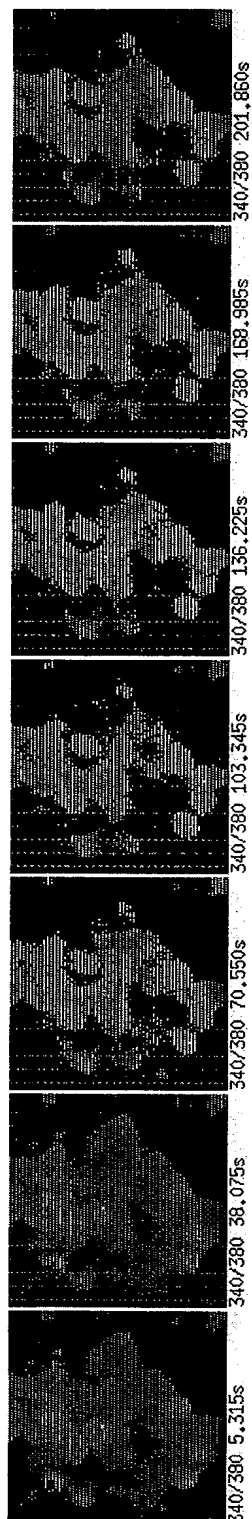


13E hVR1pCIN5 in HEK293T, 24hr transient expression, 20 min pre-incubation in 10uM capsazepine, stimulated with 10uM anandamide at time point 52 sec

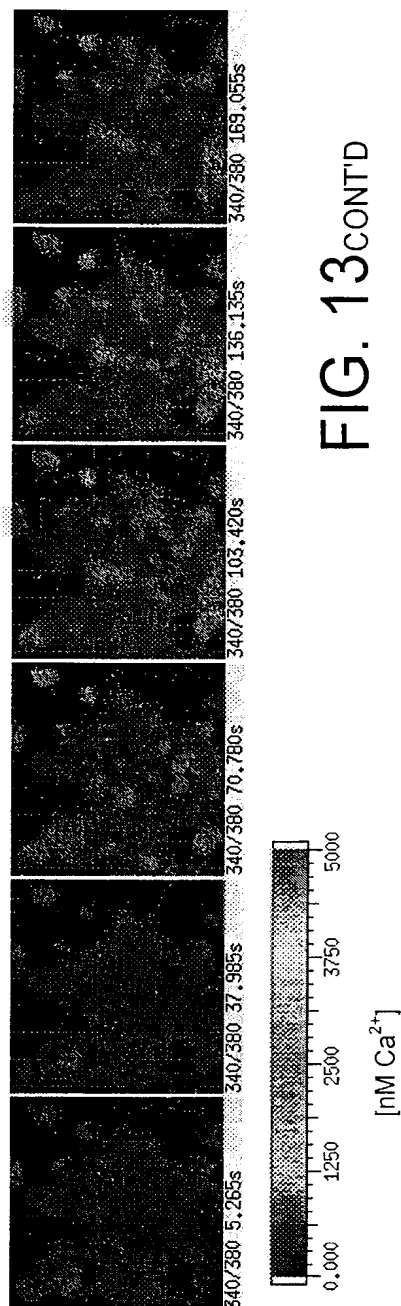
FIG. 13^{CONT'D}

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13F hVR1pCIN5 in HEK293T cells, 24hr transient expression, stimulated with 1uM Resiniferatoxin at time point 52 seconds



13G hVR1pCIN5 in HEK293T, 24hr transient expression, 20 min pre-incubation with 10 uM capsazepine, stimulated with 1 uM Resiniferatoxin at time point 52 seconds

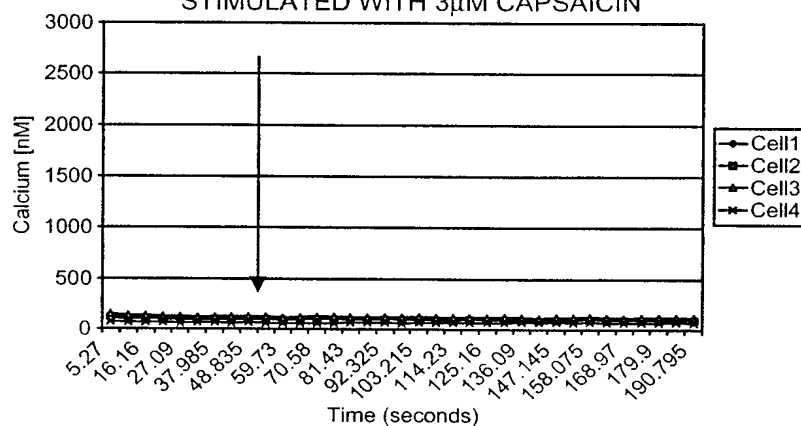
FIG. 13^{CONT'D}

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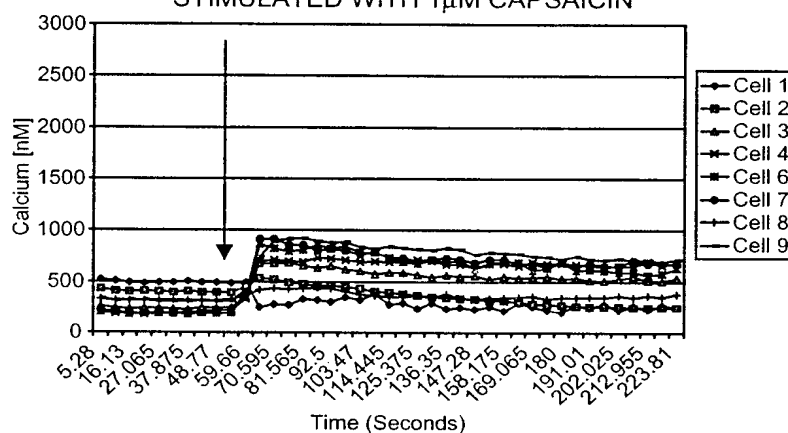
FIG. 14

EXPOSURE OF TRANSFECTED CELLS TO AGONISTS
(ADDITION INDICATED BY ARROW).

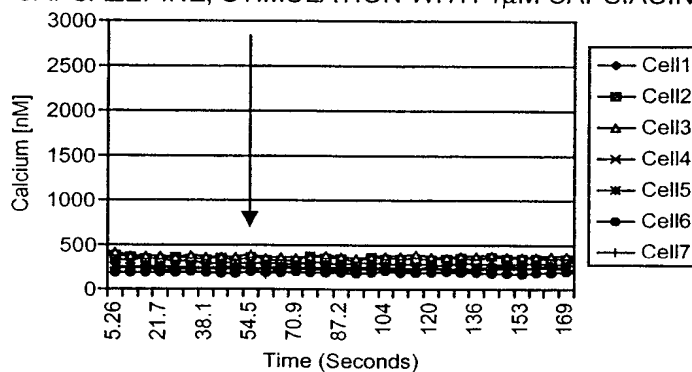
14A: pCIN5-NEW IN HEK293T, 24hr TRANSIENT EXPRESSION,
STIMULATED WITH 3 μ M CAPSAICIN



14B: hVR1pCIN5 IN HEK293T, 24hr EXPRESSION,
STIMULATED WITH 1 μ M CAPSAICIN



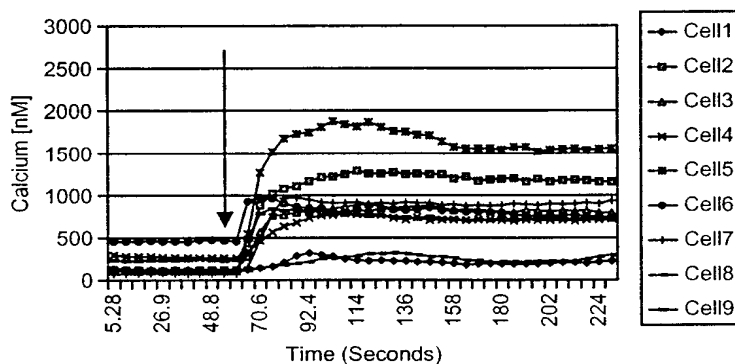
14C: hVR1pCIN5 IN HEK293T, 24hr TRANSIENT
EXPRESSION, 20 MIN PRE-INCUBATION WITH 10 μ M
CAPSAZEPINE, STIMULATION WITH 1 μ M CAPSAICIN



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FIG. 14_{CONT'D}

14D: hVR1pCIN5 IN HEK293T, 24hr TRANSIENT
EXPRESSION, STIMULATION WITH 10 μ M ANANDAMIDE



14E: hVR1pCIN5 IN HEK293T, 24hr TRANSIENT
EXPRESSION, 20 MIN PRE-INCUBATION IN 10 μ M
CAPAZEPINE, STIMULATED WITH 10 μ M ANANDAMIDE

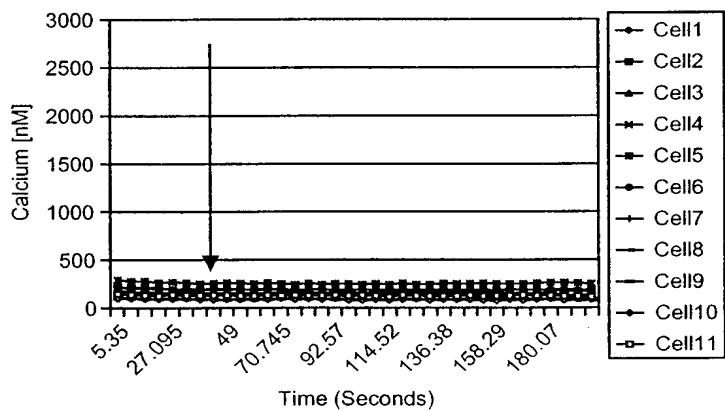
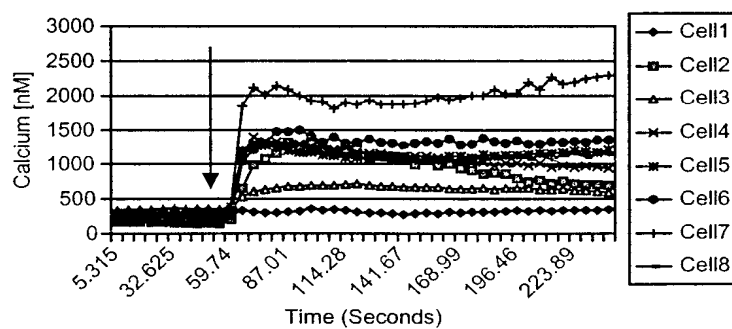
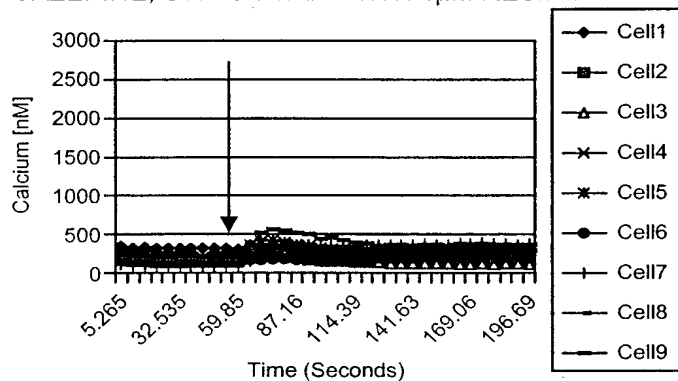


FIG. 14_{CONT'D}

14F: hVR1pCIN5 IN HEK293T CELLS, 24hr TRANSIENT
EXPRESSION, STIMULATED WITH 1 μ M RESINIFERATOXIN



14G: hVR1pCIN5 IN HEK293T, 24hr TRANSIENT
EXPRESSION, 20 MIN PRE-INCUBATION WITH 10 μ M
CAPSAZEPINE, STIMULATED WITH 1 μ M RESINIFERATOXIN



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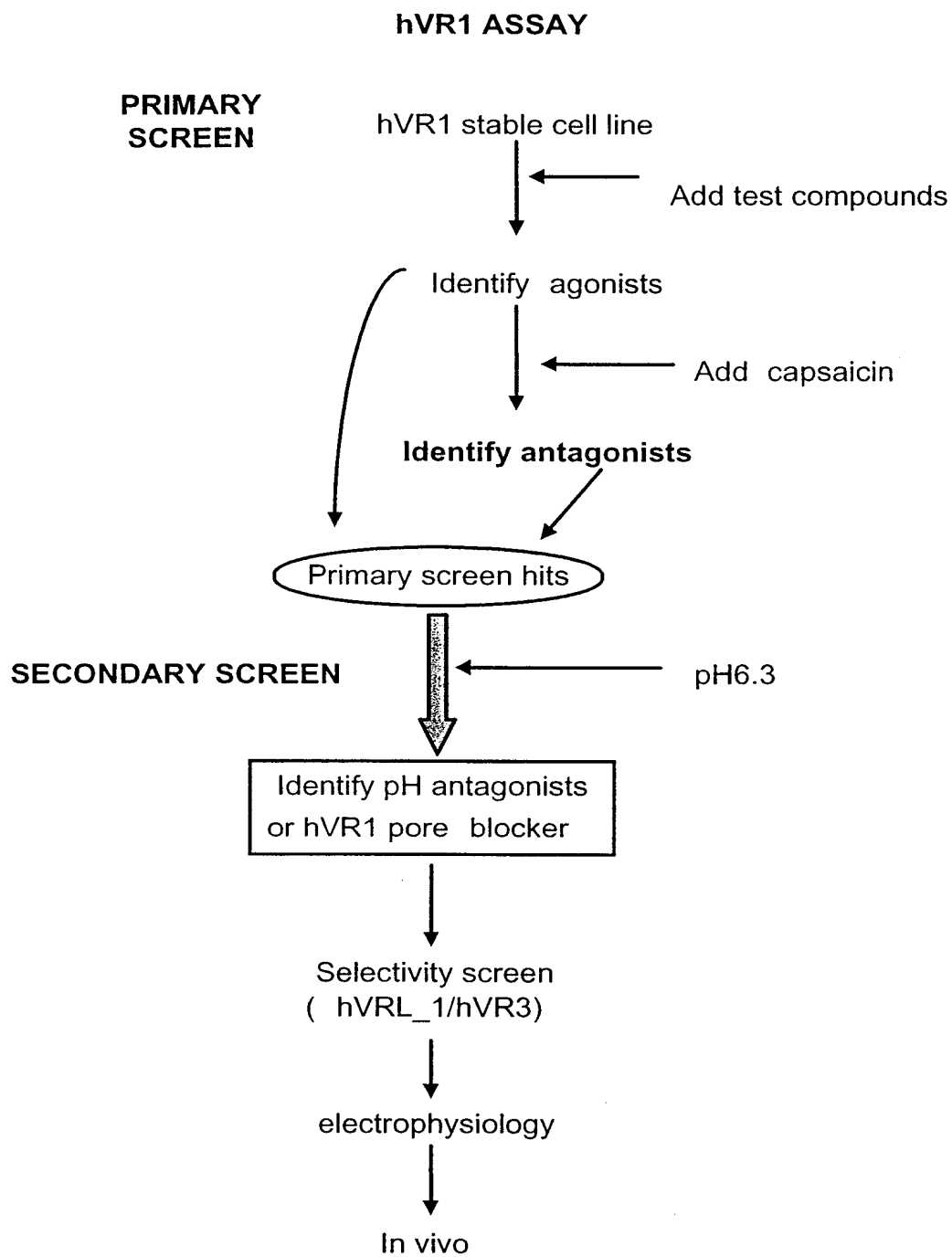
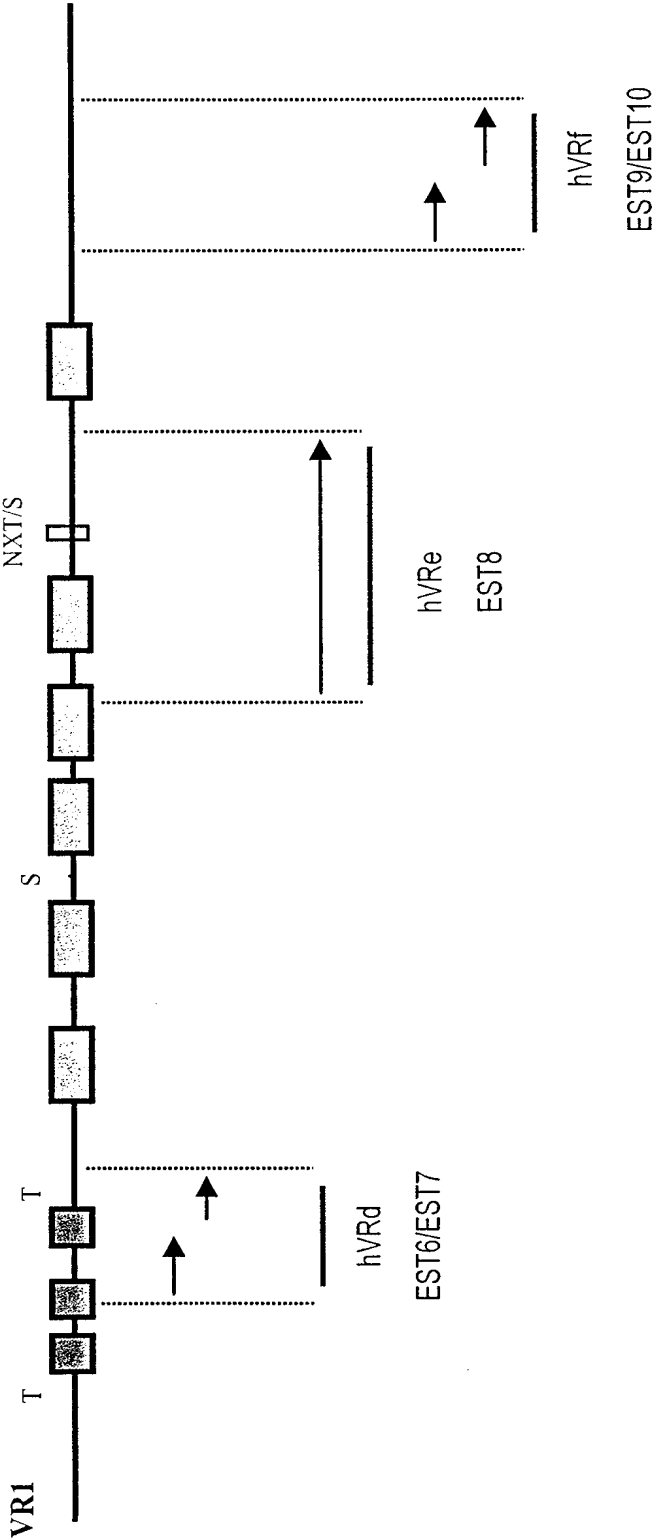


FIG. 15

FIG. 16
ALIGNMENT OF THE HUMAN VR3 IN SIL/CO CLUSTERS WITH RAT VR1



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FIG. 17

hVR3 SEQUENCE INCLUDING 5' UTR (nt -686 TO nt 0) CODING
REGION (nt1 TO nt 2889), 3'UTR (nt 2890 TO nt 3418)

-684	ttacgcgttaagaaataccacagcttatgcatcaagcttggtaccgagctcggatccact	-625
-624	agtaccgccggccagtgtgctggaattcaaggtgaggagaggagcatggatcctgggagc	-565
-564	gagtgtgtgcaggccaggaggaggctttccagaggagcccagttgagctggaacaccagtg	-505
-504	gggaggagttgaccagcaaaggtgcaggaggaggatcagcactttgcactggggagcagag	-445
-444	tttgctgactggggaagtcaactcaagtattggagcctcagtttccctgttctgtaaaatg	-385
-384	ggttcatcatgacagtgtttgatgaggaaaaggactgccggcctacacagcaagtccaca	-325
-324	tggattttctgagccctcctgtgcctgaagcccacgggttaatggttctgccttagcagg	-265
-264	tgcttaccacgtgcccaggcactgcactgcactggccactggactgcatgttctgtccatg	-205
-204	aggcttgatatcccatcttacagatcaggaagctgaggctatgaaatgtcgacttgct	-145
-144	caatgtcatggaatgactaagtgtggagcctggatttgaacttggtctctctggggctcca	-85
-84	aagctggcctttcttggtcagcagtagggtctgggatccaagtatgggggtcccagcttgac	-25
-24	cctgaagtccaccctctttcagctaaATGCCCAGGGTAGTTGGACCTGGGGCCAATTTGTG	35
36	TTTCAGGTTTCGTGAAAGAGGCTCCTGTTGCAGTTCCCGCCTGAGGCTGGCGGCCAACCA	95
96	CATCTGGGAGTGGCCTCCCTGTGCCCCCTGTCATTACAACGGTGGCTTTGAAGCAGCTGGC	155
156	AGCACTGCTGCTTGTTCCACGTGGGAGGGGGCTTCCTGGAGCCCCCGCCCCCTGGCCGGGTT	215
216	CTGCCTGACTCCCCCTTTCATTCCCTTGCAGGCTGAGCAGTGCAGACGGGCCTGGGGCAGG	275
276	CATGGCGGATTCCAGCGAAGGCCCCCGCGCGGGGCCCCGGGGAGGTGGCTGAGCTCCCCGG	335
336	GGATGAGAGTGGCACCCCAGGTGGGGAGGCTTTTCCTCTCTCCTCCCTGGCCAATCTGTT	395

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396 TGAGGGGGAGGATGGCTCCCTTTTCGCCCTCACCGGCTGATGCCAGTCGCCCTGCTGGCCC 455

456 AGGCGATGGGCGACCAAATCTGCGCATGAAGTTCCAGGGCGCCTTCCGCAAGGGGGTGCC 515

516 CAACCCCATCGATCTGCTGGAGTCCACCCTATATGAGTCCTCGGTGGTGCCTGGGCCCCAA 575

576 GAAAGCACCCATGGACTCACTGTTTGACTACGGCACCTATCGTCACCACTCCAGTGACAA 635

636 CAAGAGGTGGAGGAAGAAGATCATAGAGAAGCAGCCGCAGAGCCCCAAAGCCCCTGCCCC 695

696 TCAGCCGCCCCCATCCTCAAAGTCTTCAACCGGCCTATCCTCTTTGACATCGTGTCCCG 755

756 GGGCTCCACTGCTGACCTGGACGGGCTGCTCCCATTTCTTGCTGACCCACAAGAAACGCCT 815

816 AACTGATGAGGAGTTTCGAGAGCCATCTACGGGGAAGACCTGCCTGCCCAAGGCCTTGCT 875

876 GAACCTGAGCAATGGCCGCAACGACACCATCCCTGTGCTGCTGGACATCGCGGAGCGCAC 935

936 CGGCAACATGCGGGAGTTCATTAACCTCGCCCTTCCGTGACATCTACTATCGAGGTCAGAC 995

996 AGCCCTGCACATCGCCATTGAGCGTCGCTGCAAACACTACGTGGAATTCTCGTGGCCCA 1055

1056 GGGAGCTGATGTCCACGCCCAGGCCCGTGGGCGCTTCTTCCAGCCCAAGGATGAGGGGG 1115

1116 CTACTTCTACTTTGGGGAGCTGCCCCTGTGCTGGCTGCCTGCACCAACCAGCCCCACAT 1175

1176 TGTCAACTACCTGACGGAGAACCCCCACAAGAAGGCGGACATGCGGCGCCAGGACTCGCG 1235

1236 AGGCAACACAGTGCTGCATGCGCTGGTGGCCATTGCTGACAACACCCGTGAGAACACCAA 1295

1296 GTTTGTTACCAAGATGTACGACCTGCTGCTGCTCAAGTGTGCCCCGCTCTTCCCCGACAG 1355

1356 CAACCTGGAGGCCGTGCTCAACAACGACGGCCTCTCGCCCCCTCATGATGGCTGCCAAGAC 1415

1416 GGGCAAGATTGGGATCTTTCAGCACATCATCCGGCGGGAGGTGACGGATGAGGACACACG 1475

1476 GCACCTGTCCCGCAAGTCCAAGGACTGGGCCTATGGGCCAGTGTATTCTCGCTTTATGA 1535

FIG. 17 CONT'D

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1536 CCTCTCCTCCCTGGACACGTGTGGGGAAGAGGCCCTCCGTGCTGGAGATCCTGGTGTACAA 1595
1596 CAGCAAGATTGAGAACCGCCACGAGATGCTGGCTGTGGAGCCCATCAATGAACTGCTGCG 1655
1656 GGACAAGTGGCGGAAGTTCGGGGCCGTCTCCTTCTACATCAACGTGGTCTCCTACCTGTG 1715
1716 TGCCATGGTTATCTTCACTCTCACCGCCTACTACCAGCCGCTGGAGGGCACACCGCCGTA 1775
1776 CCCTTACCGCACCACGGTGGACTACCTGCGGCTGGCTGGCGAGGTCATTACGCTCTTCAC 1835
1836 TGGGGTCTGTCTTCTTCACTCAACATCAAAGACTTGTTTCATGAAGAAATGCCCTGGAGT 1895
1896 GAATTCTCTCTTCATTGATGGCTCCTTCCAGCTGCTCTACTTCATCTACTCTGTCTCTGGT 1955
1956 GATCGTCTCAGCAGCCCTCTACCTGGCAGGGATCGAGGCCTACCTGGCCATGATGGTCTT 2015
2016 TGCCCTGGTCTCTGGGCTGGATGAATGCCCTTTACTTCACCCGTGGGCTGAAGCTGACGGG 2075
2076 GACCTATAGCATCATGATCCAGAAGATTCTCTTCAAGGACCTTTTCCGATTCTCTGCTCGT 2135
2136 CTACTTGCTCTTCATGATCGGCTACGCTTCAGCCCTGGTCTCCCTCCTGAACCCGTGTGC 2195
2196 CAACATGAAGGTGTGCAATGAGGACCAGACCAACTGCACAGTGCCCACTTACCCCTCGTG 2255
2256 CCGTGACAGCGAGACCTTCAGCACCTTCCTCCTGGACCTGTTTAAGCTGACCATCGGCAT 2315
2316 GGGCGACCTGGAGATGCTGAGCAGCACCAGTACCCCGTGGTCTTCATCATCCTGCTGGT 2375
2376 GACCTACATCATCCTCACCTCTGTGCTGCTCCTCAACATGCTCATTGCCCTCATGGGCGA 2435
2436 GACAGTGGGCCAGGTCTCCAAGGAGAGCAAGCACATCTGGAAGCTGCAGTGGGCCACCAC 2495
2496 CATCCTGGACATTGAGCGCTCCTTCCCCGTATTCTGAGGAAGGCCTTCCGCTCTGGGGA 2555
2556 GATGGTCACCGTGGGCAAGAGCTCGGACGGCACTCCTGACCGCAGGTGGTGCTTCAGGGT 2615
2616 GGATGAGGTGAACTGGTCTCACTGGAACCAGAACTGGGCATCATCAACGAGGACCCGGG 2675

FIG. 17_{CONT'D}

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2676 CAAGAATGAGACCTACCAGTATTATGGCTTCTCGCATACCGTGGGCCGCTCCGCAGGGA 2735
2736 TCGCTGGTCCTCGGTGGTACCCCGCGTGGTGGAACTGAACAAGAACTCGAACCCGGACGA 2795
2796 GGTGGTGGTGCCTCTGGACAGCATGGGGAACCCCGCTGCGATGGCCACCAGCAGGGTTA 2855
2856 CCCCCGCAAGTGGAGGACTGATGACGCCCCGCTCtagggactgcagcccagcccagctt 2915
2916 ctctgcccactcatttctagtcocagccgcatttcagcagtgcccttctggggtgtcccccc 2975
2976 acaccctgctttggccccagagggcgagggaccagtgagggtgccagggaggccccaggac 3035
3036 cctgtggtcccctggctctgcctccccaccctggggtgggggctcccggccacctgtctt 3095
3096 gctcctatggagtcacataagccaacgccagagcccctccacctcaggccccagcccctg 3155
3156 cctctccattattttatttgctctgctctcaggaagcgacgtgaccttgccccagctgga 3215
3216 acctggcagaggccttaggaccccgttccaagtgcactgcccggccaagccccagcctca 3275
3276 gcctgcgctgagctgcatgcgccaccatttttggcagcgtggcagctttgcaaggggct 3335
3336 ggggccctcggcgtggggccatgccttctgtgtgttctgtagtgtctgggatttgccggt 3395
3396 gctcaataaatgtttattcattgaaaaaaaaaaaaaaaaa 3433

FIG. 17_{CONT'D}

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FIG. 18

NUCLEOTIDE AND AMINO ACID SEQUENCE OF hVR3
INCLUDING THE 5'UTR (nt -684 TO nt 0), CODING REGION (nt1
TO 2889) AND 3'UTR (nt 2890 TO nt 3418)

-684	ttacgcgttaagaaatacccaagcttatgcatcaagcttggtaccgagctcggatccact	-625
-624	agtaccgccggccagtggtgctggaattcaaggtgaggagaggagcatggatcctgggagc	-565
-564	gagtgtgtgcaggccaggaggaggctttccagaggagcccagttgagctggaacaccagtg	-505
-504	gggaggagttgaccagcaaaggtgcaggggagggatcagcactttgcactggggagcagag	-445
-444	tttgtgcactggggaagtcaactcaagtattggagcctcagtttctgttctgtaaaatg	-385
-384	ggttcatcatgacagtgtttgatgaggaaaaggactgccggcctacacagcaagtccaca	-325
-324	tggattttctgagccccctcctgtgcctgaagcccacggttaatggttctgccttagcagg	-265
-264	tgcttaccacgtgccaggcactgcactgcactggccactggactgcatgttctgtccatg	-205
-204	aggcttggatatccccatcttacagatcaggaagctgaggctatgaaatgtcgacttgct	-145
-144	caatgtcatggaatgactaagtgtggagocctggatttgaaacttggtctctctggggctcca	-85
-84	aagctggcttttcttggtcagcagtagggctctgggatccaagtatgggggtcccagcttgac	-25
-24	cctgaagtccaccctctttcagctaATGCCCAGGGTAGTTGGACCTGGGGCCAATTTGTG	35
1	M P R V V G P G A N L C	12
36	TTTCCAGGTTTCGTGAAAGAGGCTCCTGTTGCAGTTCCCGCCTGAGGCTGGCGGCCAACCA	95
13	F Q V R E R G S C C S S R L R L A A N H	32
96	CATCTGGGAGTGGCCTCCCTGTGCCCCCTGTCATTACAACGGTGGCTTTGAAGCAGCTGGC	155
33	I W E W P P C A P V I T T V A L K Q L A	52
156	AGCACTGCTGCTTGTCCACGTGGGAGGGGGCTTCCTGGAGCCCCCGCCCCTGGCCGGGTT	215
53	A L L L V H V G G G F L E P P P L A G F	72
216	CTGCCTGACTCCCCCTTTCATTCCCTTGCAGGCTGAGCAGTGCAGACGGGCCTGGGGCAGG	275
73	C L T P L S F P C R L S S A D G P G A G	92
276	CATGGCGGATTCCAGCGAAGGCCCCCGCGCGGGGCCCCGGGGAGGTGGCTGAGCTCCCCGG	335
93	M A D S S E G P R A G P G E V A E L P G	112
336	GGATGAGAGTGGCACCCCAGGTGGGGAGGCTTTTCTCTCTCTCCCTGGCCAATCTGTT	395
113	D E S G T P G G E A F P L S S L A N L F	132
396	TGAGGGGGAGGATGGCTCCCTTTCGCCCTCACCGGCTGATGCCAGTCGCCCTGCTGGCCC	455
133	E G E D G S L S P S P A D A S R P A G P	152
456	AGGCGATGGGCGACCAAATCTGCGCATGAAGTTCCAGGGCGCCTTCCGCAAGGGGGTGCC	515
153	G D G R P N L R M K F Q G A F R K G V P	172
516	CAACCCCATCGATCTGCTGGAGTCCACCCTATATGAGTCCTCGGTGGTGCCTGGGCCCAA	575
173	N P I D L L E S T L Y E S S V V P G P K	192

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576	GAAAGCACCCATGGACTCACTGTTTGACTACGGCACCTATCGTCACCACTCCAGTGACAA	635
193	K A P M D S L F D Y G T Y R H H S S D N	212
636	CAAGAGGTGGAGGAAGAAGATCATAGAGAAGCAGCCGAGAGCCCCAAAGCCCCTGCCCC	695
213	K R W R K K I I E K Q P Q S P K A P A P	232
696	TCAGCCGCCCCCATCCTCAAAGTCTTCAACCGGCCTATCCTCTTTGACATCGTGTCCCG	755
233	Q P P P I L K V F N R P I L F D I V S R	252
756	GGGCTCCACTGCTGACCTGGACGGGCTGCTCCCATTCTTGCTGACCCACAAGAAACGCCT	815
253	G S T A D L D G L L P F L L T H K K R L	272
816	AACTGATGAGGAGTTTCGAGAGCCATCTACGGGGAAGACCTGCCTGCCCAAGGCCTTGCT	875
273	T D E E F R E P S T G K T C L P K A L L	292
876	GAACCTGAGCAATGGCCGCAACGACACCATCCCTGTGCTGCTGGACATCGCGGAGCGCAC	935
293	N L S N G R N D T I P V L L D I A E R T	312
936	CGGCAACATGCGGGAGTTCATTAACTCGCCCTTCCGTGACATCTACTATCGAGGTCAGAC	995
313	G N M R E F I N S P F R D I Y Y R G Q T	332
996	AGCCCTGCACATCGCCATTGAGCGTCGCTGCAAACACTACGTGGAACCTTCTCGTGGCCCA	1055
333	A L H I A I E R R C K H Y V E L L V A Q	352
1056	GGGAGCTGATGTCCACGCCCAGGCCCGTGGGCGCTTCTTCCAGCCCAAGGATGAGGGGGG	1115
353	G A D V H A Q A R G R F F Q P K D E G G	372
1116	CTACTTCTACTTTGGGGAGCTGCCCCCTGTCGCTGGCTGCCTGCACCAACCAGCCCCACAT	1175
373	Y F Y F G E L P L S L A A C T N Q P H I	392
1176	TGTCAACTACCTGACGGAGAACCCCCACAAGAAGGCGGACATGCGGCGCCAGGACTCGCG	1235
393	V N Y L T E N P H K K A D M R R Q D S R	412
1236	AGGCAACACAGTGTGCTGCATGCGCTGGTGGCCATTGCTGACAACACCCGTGAGAACACCAA	1295
413	G N T V L H A L V A I A D N T R E N T K	432
1296	GTTTGTATTACCAAGATGTACGACCTGCTGCTGCTCAAGTGTGCCCGCCTCTTCCCCGACAG	1355
433	F V T K M Y D L L L L K C A R L F P D S	452
1356	CAACCTGGAGGCCGTGCTCAACAACGACGGCCTCTCGCCCCTCATGATGGCTGCCAAGAC	1415
453	N L E A V L N N D G L S P L M M A A K T	472
1416	GGGCAAGATTGGGATCTTTCAGCACATCATCCGGCGGGAGGTGACGGATGAGGACACACG	1475
473	G K I G I F Q H I I R R E V T D E D T R	492
1476	GCACCTGTCCCGCAAGTCCAAGGACTGGGCCTATGGGCCAGTGATTCTCTCGCTTTATGA	1535
493	H L S R K S K D W A Y G P V Y S S L Y D	512
1536	CCTCTCCTCCCTGGACACGTGTGGGGAAGAGGCCTCCGTGCTGGAGATCCTGGTGTACAA	1595
513	L S S L D T C G E E A S V L E I L V Y N	532
1596	CAGCAAGATTGAGAACCGCCACGAGATGCTGGCTGTGGAGCCCATCAATGAACTGCTGCG	1655
533	S K I E N R H E M L A V E P I N E L L R	552
1656	GGACAAGTGGCGGAAGTTCGGGGCCGTCTCCTTCTACATCAACGTGGTCTCCTACCTGTG	1715
553	D K W R K F G A V S F Y I N V V S Y L C	572

FIG. 18_{CONT'D}

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1716	TGCCATGGTTATCTTCACTCTCACCGCCTACTACCAGCCGCTGGAGGGCACACCGCCGTA	1775
573	A M V I F T L T A Y Y Q P L E G T P P Y	592
1776	CCCTTACCGCACCACGGTGGACTACCTGCGGCTGGCTGGCGAGGTCATTACGCTCTTCAC	1835
593	P Y R T T V D Y L R L A G E V I T L F T	612
1836	TGGGGTCCTGTTCTTCTTACCAACATCAAAGACTTGTTTCATGAAGAAATGCCCTGGAGT	1895
613	G V L F F F T N I K D L F M K K C P G V	632
1896	GAATTCTCTCTTCATTGATGGCTCCTTCCAGCTGCTCTACTTCATCTACTCTGTCCTGGT	1955
633	N S L F I D G S F Q L L Y F I Y S V L V	652
1956	GATCGTCTCAGCAGCCCTCTACCTGGCAGGGATCGAGGCCTACCTGGCCATGATGGTCTT	2015
653	I V S A A L Y L A G I E A Y L A M M V F	672
2016	TGCCCTGGTCCTGGGCTGGATGAATGCCCTTTACTTCACCCGTGGGCTGAAGCTGACGGG	2075
673	A L V L G W M N A L Y F T R G L K L T G	692
2076	GACCTATAGCATCATGATCCAGAAGATTCTCTTCAAGGACCTTTTCCGATTCTGCTCGT	2135
693	T Y S I M I Q K I L F K D L F R F L L V	712
2136	CTACTTGCTCTTCATGATCGGCTACGCTTCAGCCCTGGTCTCCCTCCTGAACCCGTGTGC	2195
713	Y L L F M I G Y A S A L V S L L N P C A	732
2196	CAACATGAAGGTGTGCAATGAGGACCAGACCAACTGCACAGTGCCCACTTACCCCTCGTG	2255
733	N M K V C N E D Q T N C T V P T Y P S C	752
2256	CCGTGACAGCGAGACCTTCAGCACCTTCCTCCTGGACCTGTTTAAGCTGACCATCGGCAT	2315
753	R D S E T F S T F L L D L F K L T I G M	772
2316	GGGCGACCTGGAGATGCTGAGCAGCACCAAGTACCCCGTGGTCTTCATCATCCTGCTGGT	2375
773	G D L E M L S S T K Y P V V F I I L L V	792
2376	GACCTACATCATCCTCACCTCTGTGCTGCTCCTCAACATGCTCATTGCCCTCATGGGCGA	2435
793	T Y I I L T S V L L L N M L I A L M G E	812
2436	GACAGTGGGCCAGGTCTCCAAGGAGAGCAAGCACATCTGGAAGCTGCAGTGGGCCACCAC	2495
813	T V G Q V S K E S K H I W K L Q W A T T	832
2496	CATCCTGGACATTGAGCGCTCCTTCCCCGTATTCTGAGGAAGGCCTTCCGCTCTGGGGA	2555
833	I L D I E R S F P V F L R K A F R S G E	852
2556	GATGGTCACCGTGGGCAAGAGCTCGGACGGCACTCCTGACCGCAGGTGGTGCTTCAGGGT	2615
853	M V T V G K S S D G T P D R R W C F R V	872
2616	GGATGAGGTGAACTGGTCTCACTGGAACCAGAACTTGGGCATCATCAACGAGGACCCGGG	2675
873	D E V N W S H W N Q N L G I I N E D P G	892
2676	CAAGAATGAGACCTACCAGTATTATGGCTTCTCGCATACCGTGGGCGCCTCCGCAGGGA	2735
893	K N E T Y Q Y Y G F S H T V G R L R R D	912
2736	TCGCTGGTCTCGGTGGTACCCCGGTGGTGGAACTGAACAAGAACTCGAACCCGGACGA	2795
913	R W S S V V P R V V E L N K N S N P D E	932
2796	GGTGGTGGTGCCTCTGGACAGCATGGGGAACCCCGCTGCGATGGCCACCAGCAGGGTTA	2855
933	V V V P L D S M G N P R C D G H Q Q G Y	952

FIG. 18_{CONT'D}

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2856	CCCCCGCAAGTGGAGGACTGATGACGCCCCGCTCtagggactgcagcccagccccagctt	2915
953	P R K W R T D D A P L	963
2916	ctctgcccactcattttctagtcacagccgcattttcagcagtgcccttctggggtgtcccccc	2975
2976	acaccctgctttggccccagagggcgagggaccagtgagggtgccagggaggccccaggac	3035
3036	cctgtggtcccctggctctgcctccccaccctggggtgggggctcccggccacctgtctt	3095
3096	gtccctatggagtcacataagccaacgccagagcccctccacctcaggccccagcccctg	3155
3156	cctctccattattttatttgctctgctctcaggaagcgacgtgaccctgccccagctgga	3215
3216	acctggcagaggccttaggaccccgttccaagtgcactgcccggccaagccccagcctca	3275
3276	gcctgcgcctgagctgcatgcgccaccatttttggcagcgtggcagctttgcaaggggt	3335
3336	ggggccctcggcgtggggccatgccttctgtgtgttctgtagtgtctgggatttgccggt	3395
3396	gtcaataaaatgtttattcattgaaaaaaaaaaaaaaaaa	3433

FIG. 18_{CONT'D}


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FIG. 19

AMINO ACID SEQUENCE OF hVR3

1 MPRVVGPAN LCFQVRERGS CCSSRLRLAA NHIWEWPPCA PVITTVALKQ
 51 LAALLLVHVG GGFLEPPPLA GFCLTPLSFP CRLSSADGPG AGMADSSEGP
 101 RAGPGEVAEL PGDESGTPGG EAFPLSSLAN LFEGEDGSLS PSPADASRPA
 151 GPGDGRPNLR MKFQGAFRKG VPNPIDLLES TLYESSVVPK PKKAPMDSL
 201 DYGTYRHHSS DNKRWRKKII EKQPQSPKAP APQPPPIKV FNRPIFLDIV
 251 SRGSTADLDG LLPFLLTHKK RLTDEEFREP STGKTCLPKA LLNLSNGRND
 301 TIPVLLDIAE RTGNMREFIN SPFRDIYYRG QTALHIAIER RCKHYVELLV
 351 AQGADVHAQA RGRFFQPKDE GGYFYFEGELP LSLAACTNQP HIVNYLTENP
 401 HKKADMRRQD SRGNTVLHAL VAIADNTREN TKFVTKMYDL LLLKCARLFP
 451 DSNLEAVLNN DGLSPILMAA KTGKIGIFQH IIRREVTDED TRHLSRKS
 501 WAYGPVYSSL YDLSSLDTCG EEASVLEILV YNSKIENRHE MLAVEPINEL
 551 LRDKWRKFGA VSEVINVWSY ICAMVIEITITAYYQPLEGTP PYPYRTTVDY
 601 LRLAGEVITITITCMTTEFTN IKDLFMKKCP GVNSLFDGSE FOLLYEITYSV
 651 IVIVSAALYD AGIEAYLAMM VFALVIGWMN ALYETRGLKI TCTYSIMIK
 701 ILFKDIEFTLIVYILFMIGYASALVSLINP CANMKVCNED QTNCTVPTYP
 751 SCRDSSETFST FLDLFKLTI GMDLEMLSS TKPVPVEIIL LVYITITTSV
 801 ILLNMITATLMGETVGQVSKE SKHIWKLQWA TTILDIERSE PVFLRKAFRS
 851 GEMVTVGKSS DGTPDRRWCF RVDEVNWSHW NQNLGIINED PGKNETYQYY
 901 GFSHTVGRLR RDRWSSVVR VVELNKN SNP DEVVVPDLSM GNPRCDGHQQ
 951 GYPRKWRTDD APL

Key

 Transmembrane domains


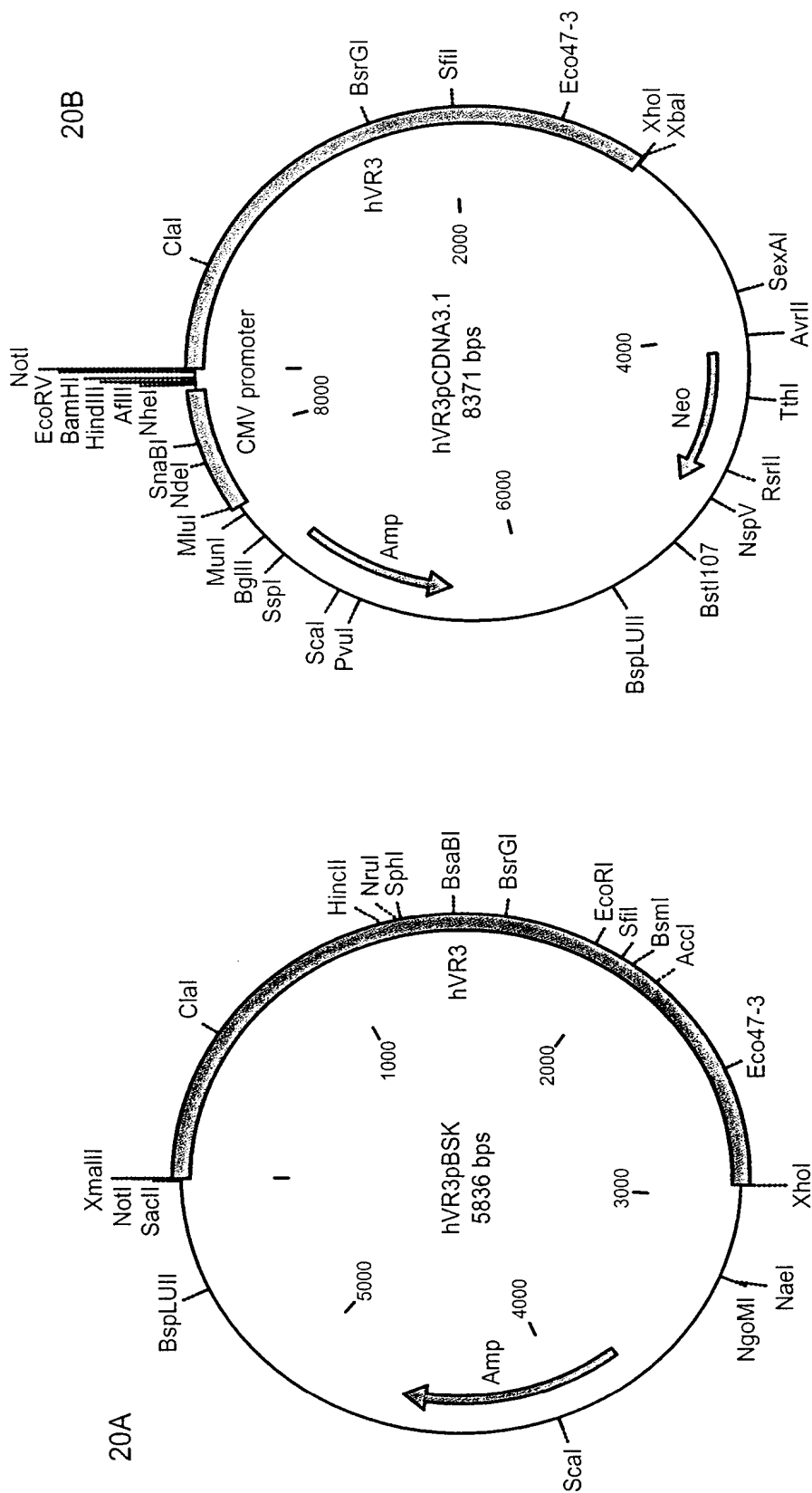
 Ankyrin binding domains

FIG. 20

FULL-LENGTH hVR3 CLONED INTO (A) pBLUESCRIPT SK(+) (hVR3pBSK) AND
(B) pCDNA3.1(+) (hVR1pCDNA3.1) VIA NotI/XhoI RESTRICTION SITES.



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FIG. 21

A MULTIPLE COMPARISON OF THE AMINO ACID SEQUENCES OF THE RAT VR1 AND THE HUMAN VANILLOID RECEPTORS, hVR1, hVRL-1 AND hVR3

	10	20	30	40	50
VR1	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~
hVR1	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~
hVRL-1	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~
hVR3	MPRVVGPANLCFQVRERGGSCSSRLRLAANHIWEWPPCAPVITTVALKQ				
	60	70	80	90	100
VR1	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~
hVR1	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~
hVRL-1	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~
hVR3	LAALLLVHVGGGFLEPPPLAGFCLTPLSFPCRLSSADGPGAGMADSSEGP				
	110	120	130	140	150
VR1	~~~~~	~~~~~	MEQRASLDSEESESPPOENSCL	~~~~~	~~~~~
hVR1	~~~~~	~~~~~	MKKWSSTDLGAAADPLQKDTCP	~~~~~	~~~~~
hVRL-1	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~
hVR3	RAGPGEVAELPGDESGTPGGEAFPLSSLANLFEGEDGSLSPSPADASRPA				
	160	170	180	190	200
VR1	DPPDRDPNCKPPPVPKPHIFTTRSRTLFG...KGDSEEASPLDCPYEEG				
hVR1	DPLDGDPSRPPPAKPOLSTAKSRTLFG...KGDSEEAFVDCPHEEG				
hVRL-1	~~~~~	~~~~~	MTSPSSSPVFRLETLDGGQEDGSEADRGKLD	~~~~~	~~~~~
hVR3	GPGDGRPNLRMKFQGAFRKGVFNP...IDLLESTLYESSVVPGPKKAP				
	210	220	230	240	250
VR1	GLASCPITVSSVLTIQRPDGPASVRPSSQDSVSAG.EKP.PRLYDRRS				
hVR1	ELDSCPTITVSPVITIQRPDGGPTGARLLSQDSVAASTEKT.LRLYDRRS				
hVRL-1	GSGLPFM..ESQFQGEDRKFAPOIRVNLNRYKGTGASQPD.P.NR.FDRDR				
hVR3	MDSLFDYGTYRHHSSDNKRWRKKIIEKQPQSPKAPAPQPPPIKVFNRP				
	260	270	280	290	300
VR1	IFDAVAOSNCOELESLLPFLQSKKRLTDSEFKDPETGKTCLLKAMLNH				
hVR1	IFEAVAQNNCODLESLLLFLOKSKKHLTDNEFKDPETGKTCLLKAMLNH				
hVRL-1	LENVSRGVPEDLAGLPEYLSKTSKYLTDSYTEGSTGKTCIMKAVLNK				
hVR3	LFDIVSRGSTADLDGLLPFLTHKKRLTDEEFREPSGKTCIPKALLNLS				
	310	320	330	340	350
VR1	NGQNDTIALLLDVARKTDSLKQFVNASYTDSYKGTALHIAIERRNMTL				
hVR1	DGQNTTIPILLEIARQTDSELKELVNASYTDSYKGTALHIAIERRNMTL				
hVRL-1	DGVNACILPLLQIDRDSGNPQPLVNAQCTDDYYRGHSALHIAIEKRSLOC				
hVR3	NGRNDTIPVLLDIAERTGNMREFINSFPRDIYYRGQALHIAIERRCKHY				
	360	370	380	390	400
VR1	VTLLVENGADVOAAANGDFFKKTKGRPGFYFGEPLPSLAACNTQLAIVKF				
hVR1	VTLLVENGADVOAAAHGDFFKKTKGRPGFYFGEPLPSLAACNTQLGIVKF				
hVRL-1	VKLLVENGANVHARACGRFFQKQG.TCFYFGEPLPSLAACKQWDVVS				
hVR3	VELLVAGADVHAQARGREFQPKDEGGYFGEPLPSLAACNTQPHIVNY				
	410	420	430	440	450
VR1	LLONSWQPADISARDSVGNTVLHALVEVADNTVDNTKFTVSMYNEILILG				
hVR1	LLONSWQTADISARDSVGNTVLHALVEVADNTADNTKFTVSMYNEILILG				
hVRL-1	LLENPHQASLQATDSQNTVLHALVMSDNSAENIALVTSMYDGLLQAG				
hVR3	LTENPHKKADMRRQDSRGNTVLHALVAIADNTRENTKFTVTKMYDLLLLKC				
	460	470	480	490	500
VR1	AKLHP TLKLEEITNRKGLTPLALAASSGKIGVLAYILOREIHEPECRLHS				
hVR1	AKLHP TLKLEELTNKGMTPLALAAGTGKIGVLAYILOREIOEPECRLHS				
hVRL-1	ARLCPTVQLEDIRNLQDLTPLKLAKEGKIEIFRHILOREFS..GLSHES				
hVR3	ARLFFDSNLEAVLNNDGLSPLMAAKTGKIGIFQHIIRREVTDEDTRHS				

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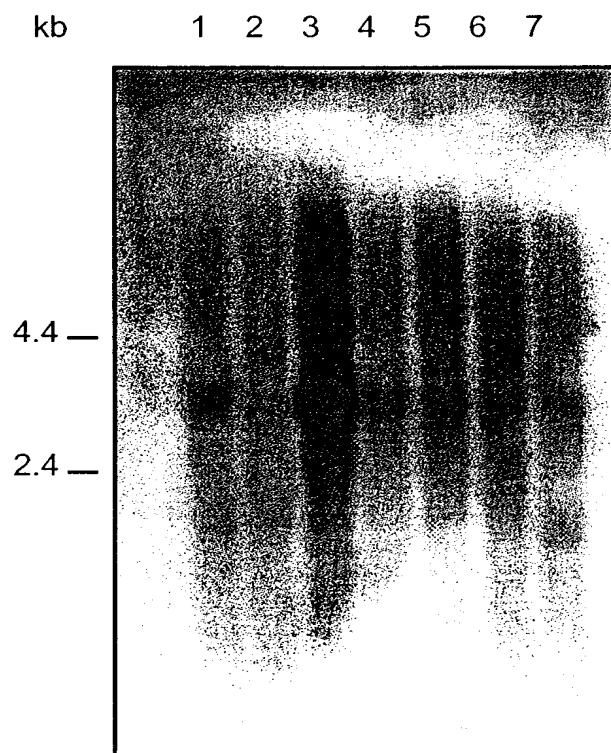
	510	520	530	540	550
VR1	RKFT	EWAY	GPVH	SSLY	DLSCIDTC.EKNSVLEVIAYSSSETPNRHDMLLV
hVR1	RKFT	EWAY	GPVH	SSLY	DLSCIDTC.EKNSVLEVIAYSSSETPNRHDMLLV
hVRL-1	RKFT	EW	CGPVR	VS	SLYDLASVDSC.EENSVLEIIAF.HCKSPHRHRMVVL
hVR3	RKSK	WAY	GPVY	SSLY	DLSSLDTCGEEASVLEILVY.WSKIENRHEMLAV
	560	570	580	590	600
VR1	EPLNRLLQDKWDRFVKRIF	YFNFFVYCLYMIIFTAAAYYRPV..EGLPPY			
hVR1	EPLNRLLQDKWDRFVKRIF	YFNFLVYCLYMIIFTMAAYYRPV..DGLPPF			
hVRL-1	EPLNKLQAKWDL	LIPK.FFLNFLCNLIYMFIFTAVAYHQPTLKKQAAPH			
hVR3	EPINELLRD	KWRKFGAVSEYINVVS	YLCAMVIFTLTAYYQPL..EGTPPY		
	610	620	630	640	650
VR1	KLKNTVGDYFRVTGEILSV	SGGVYFFFRGIQ.YFLQRRPSLKSLEFVDSYS			
hVR1	KMEN.IGDYFRVTGEILSV	LGGVYFFFRGIQ.YFLQRRPSMKTLEFVDSYS			
hVRL-1	LNAEVGNSMLLTGHIL	ILLGGIYLLVGQLW.YFWRRHVFIWISFIDS			
hVR3	PYRTTV.DYLR	LAGEVITLFTGVLEFFFTNIKDLFMKKCPGVNSLEIDGSF			
	660	670	680	690	700
VR1	EILFFVQSLF	MLVSVVLYFSQRKEYVASMVFS	LAMGWTNMLYYTRGFQOM		
hVR1	EMLFFLQSLF	MLATVVLYF	SHLKEYVASMVFS	LALGWTNMLYYTRGFQOM	
hVRL-1	EILFLFQALLTVVS	QVLCFLAIEWYLP	LLVSALVGLWLNLLYYTRGFQHT		
hVR3	QLLYFIYSVLVIV	SAALYLAGIEAYLAMV	FALVGLWMNALYFTRGLKLT		
	710	720	730	740	750
VR1	GIYAVMIEKMILRDLC	REFMEVYLVFLFGFSTAVVT	LIEDGKNNSLP....		
hVR1	GIYAVMIEKMILRDLC	REFMEVYIVFLFGFSTAVVT	LIEDGKNDSLIP....		
hVRL-1	GIYSVMIOKVILRD	LLRFLLIYLVFLFGFAVALV	SLSQEAWRPEAPTGN		
hVR3	GTYSIMIQKILFKDL	FRFLVYLLFMIGYASALV	SLLNPCANMKVCNEDQ		
	760	770	780	790	800
VR1	MESTPHKCRGSACK.	PGNSYNSLYSTCLEL	FKFTIGMGDLEFTENYDFKA		
hVR1	SESTSHRWGPACRPPD	SSYNSLYSTCLEL	FKFTIGMGDLEFTENYDFKA		
hVRL-1	ATESVQPMEGQED	EGNGAQYRGILEASLEL	FKFTIGMGELAQEQQLHFRG		
hVR3	TNCTVPTY..PSCR.	DSETFSTFL...LDL	FKLTIGMGDLEMLSSTKYPV		
	810	820	830	840	850
VR1	VEIILLAYVILTYI	ILLNMLIALMGETV	NKIAQESKNIWK	LQRAITILD	
hVR1	VEIILLAYVILTYI	ILLNMLIALMGETV	NKIAQESKNIWK	LQRAITILD	
hVRL-1	MVILLILAYVLLTYI	ILLNMLIALMSETV	NSVATDSWSIWKLQK	KAISVLE	
hVR3	VEIILLVITYIILT	SVLLNMLIALMGETV	GQVSKE SKHIWK	LQWATTILD	
	860	870	880	890	900
VR1	TEKSEFLKCMRKA	FRSGKLLQVGFTPDG	KDDYRWC	FRVDEVNWT	TTWNTNVG
hVR1	TEKSEFLKCMRKA	FRSGKLLQVGFTPDG	KDDYRWC	FRVDEVNWT	TTWNTNVG
hVRL-1	MENGYWWC.RKKQ	RAGVMLTVG	TKPDGSPDERWC	FRVEEVN	WASWEQTLF
hVR3	IERSFPVFLRKA	FRSGEMVTVGKSSDGT	PDRRWC	FRVDEVN	WNSHWNQNLG
	910	920	930	940	950
VR1	IINEDPGNCE.....	GVKRTLSESLRSG.....	RVSGRNWKNFALV		
hVR1	IINEDPGNCE.....	GVKRTLSESLRSS.....	RVSGRHWKNFALV		
hVRL-1	TLCEDPSGA.....	GVPRTLENPVLAS.....	PPKEDEDGASEENYVPV		
hVR3	IINEDPGKWETYQ	YGFSTVGR	LRDRWSSV	PRVVELN	KNSNPDEVVV
	960	970	980	990	
VR1	PLLRDASTRDRHATQ	QEEVQLKHYTGSLK	PEDAEVFKDS	SMVPGEN	
hVR1	PLLRDASTRDRHATQ	QEEVQLKHYTGSLK	PEDAEVFKDS	SMVPGEN	
hVRL-1	QLLQSN~~~~~				
hVR3	PLDSMGNPRCDGHQ	QGYPRKWRTDDAPL~~~~~			

FIG. 21 CONT'D

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FIG. 22A

HYBRIDISATION OF A NORTHERN BLOT WITH hVR3



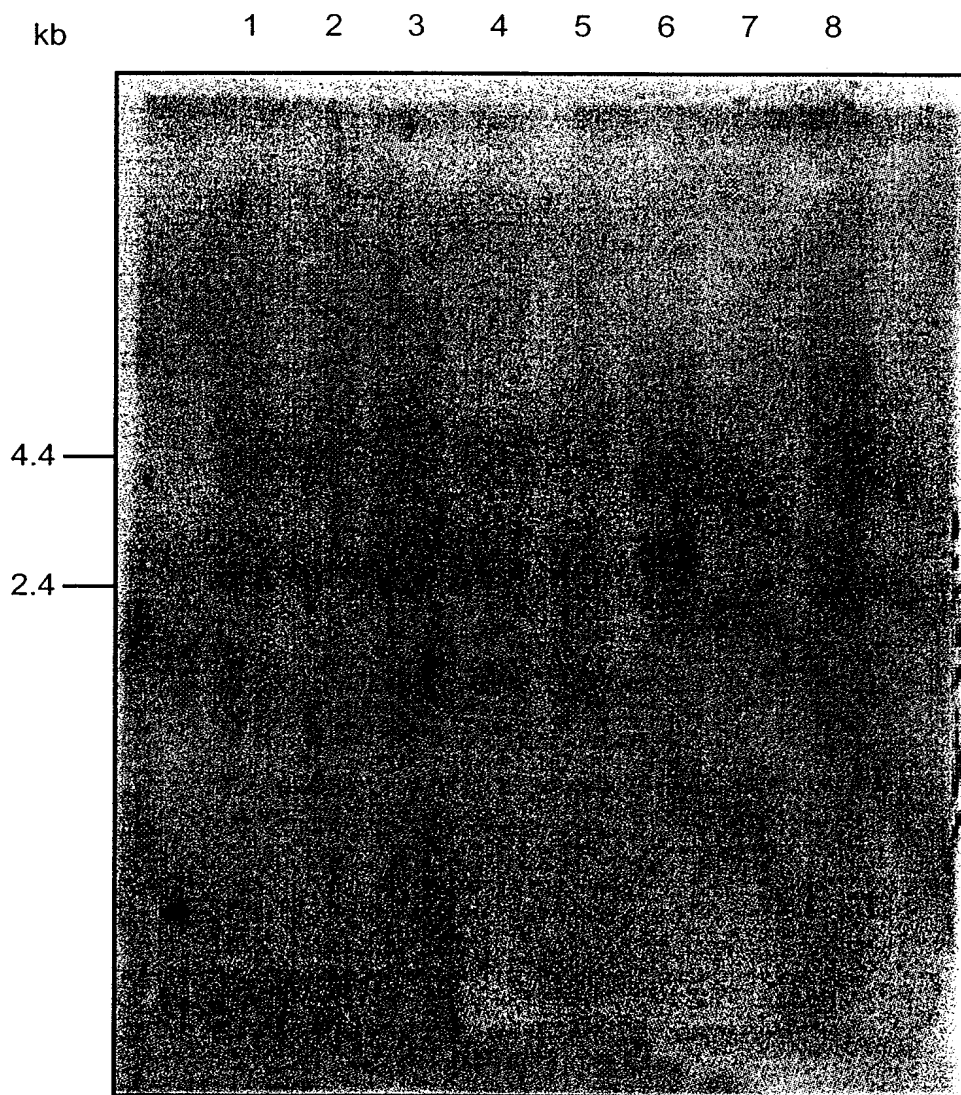
LANE 1: BONE MARROW
LANE 2: ADRENAL GLAND
LANE 3: TRACHEA
LANE 4: LYMPH NODE

LANE 5: SPINAL CORD
LANE 6: THYROID
LANE 7: STOMACH

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FIG. 22B

HYBRIDISATION OF NORTHERN BLOT WITH hVR3 PROBE



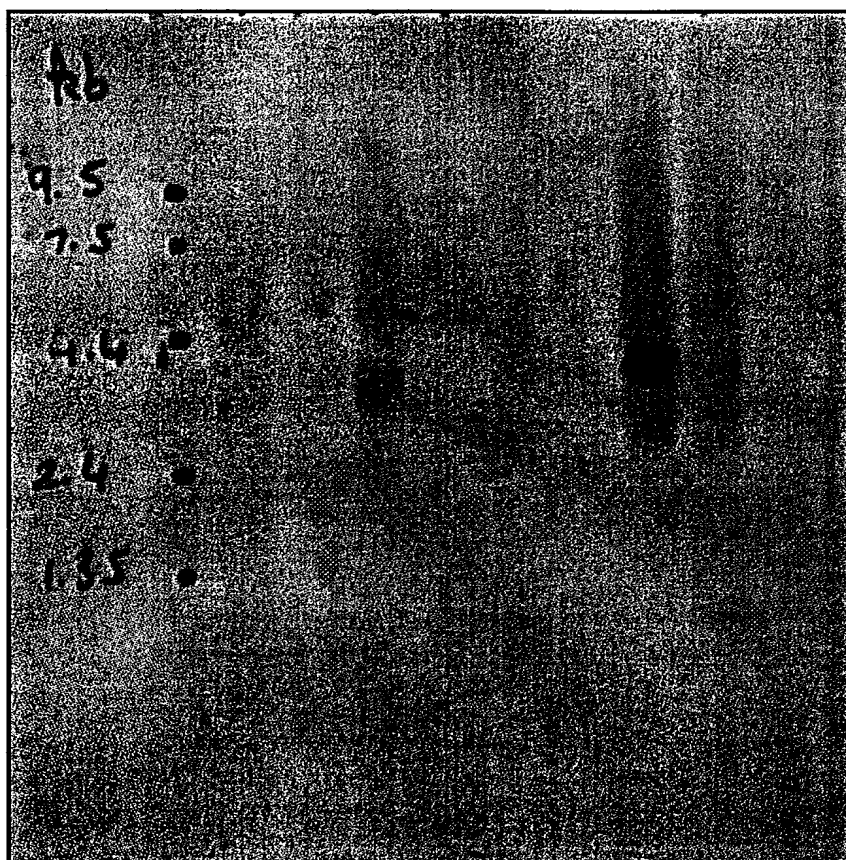
LANE 1: PERIPHERAL BLOOD
LEUKOCYTE
LANE 2: COLON
LANE 3: SMALL INTESTINE
LANE 4: UTERUS
LANE 5: TESTIS
LANE 6: PROSTATE
LANE 7: THYROID
LANE 8: SPLEEN

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FIG. 22C

HYBRIDISATION OF A MULTI-TISSUE NORTHERN
BLOT WITH THE hVR3 PROBE

1 2 3 4 5 6 7 8



LANE 1: HEART
LANE 2: BRAIN
LANE 3: PLACENTA
LANE 4: LUNG

LANE 5: LIVER
LANE 6: SKELETAL MUSCLE
LANE 7: KIDNEY
LANE 8: PANCREAS

SEQUENCE LISTING

<110> Glaxo Group Ltd
Tate, Simon N
Delany, Natalie S
Sanseau, P

<120> Novel Receptors

<130> PG3606

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<150> GB 9826359.3

<151> 1998-12-01

<160> 40

<170> PatentIn Ver. 2.1

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tgtataagct cagtggctgt ggcagcgagg ttgaagagca aaggcaggcc gggcacctgg 180

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ccggcgtggg ggctgctgca ggttgcacac tgggccacag aggatccagc aagg atg 777

Met

1

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Lys Lys Trp Ser Ser Thr Asp Leu Gly Ala Ala Ala Asp Pro Leu Gln

5

10

15

aag gac acc tgc cca gac ccc ctg gat gga gac cct aac tcc agg cca 873
Lys Asp Thr Cys Pro Asp Pro Leu Asp Gly Asp Pro Asn Ser Arg Pro

20

25

30

cct cca gcc aag ccc cag ctc tcc acg gcc aag agc cgc acc cgg ctc 921
Pro Pro Ala Lys Pro Gln Leu Ser Thr Ala Lys Ser Arg Thr Arg Leu

35

40

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50

55

60

65

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Ile Thr Ile Gln Arg Pro Gly Asp Gly Pro Thr Gly Ala Arg Leu Leu	
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Asp Asn Glu Phe Lys Asp Pro Glu Thr Gly Lys Thr Cys Leu Leu Lys	
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Ala Met Leu Asn Leu His Asp Gly Gln Asn Thr Thr Ile Pro Leu Leu	
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Leu Glu Ile Ala Arg Gln Thr Asp Ser Leu Lys Glu Leu Val Asn Ala	
180 185 190	
agc tac acg gac agc tac tac aag ggc cag aca gca ctg cac atc gcc	1401
Ser Tyr Thr Asp Ser Tyr Tyr Lys Gly Gln Thr Ala Leu His Ile Ala	
195 200 205	

atc gag aga cgc aac atg gcc ctg gtg acc ctc ctg gtg gag aac gga 1449
 Ile Glu Arg Arg Asn Met Ala Leu Val Thr Leu Leu Val Glu Asn Gly
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gca gac gtc cag gct gcg gcc cat ggg gac ttc ttt aag aaa acc aaa 1497
 Ala Asp Val Gln Ala Ala Ala His Gly Asp Phe Phe Lys Lys Thr Lys
 230 235 240

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 Gly Arg Pro Gly Phe Tyr Phe Gly Glu Leu Pro Leu Ser Leu Ala Ala
 245 250 255

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 Cys Thr Asn Gln Leu Gly Ile Val Lys Phe Leu Leu Gln Asn Ser Trp
 260 265 270

cag acg gcc gac atc agc gcc agg gac tcg gtg ggc aac acg gtg ctg 1641
 Gln Thr Ala Asp Ile Ser Ala Arg Asp Ser Val Gly Asn Thr Val Leu
 275 280 285

cac gcc ctg gtg gag gtg gcc gac aac acg gcc gac aac acg aag ttt 1689
 His Ala Leu Val Glu Val Ala Asp Asn Thr Ala Asp Asn Thr Lys Phe
 290 295 300 305

gtg acg agc atg tac aat gag att ctg atc ctg ggg gcc aaa ctg cac 1737
 Val Thr Ser Met Tyr Asn Glu Ile Leu Ile Leu Gly Ala Lys Leu His
 310 315 320

ccg acg ctg aag ctg gag gag ctc acc aac aag aag gga atg acg ccg 1785
 Pro Thr Leu Lys Leu Glu Glu Leu Thr Asn Lys Lys Gly Met Thr Pro
 325 330 335

ctg gct ctg gca gct ggg acc ggg aag atc ggg gtc ttg gcc tat att 1833
 Leu Ala Leu Ala Ala Gly Thr Gly Lys Ile Gly Val Leu Ala Tyr Ile
 340 345 350

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Leu Gln Arg Glu Ile Gln Glu Pro Glu Cys Arg His Leu Ser Arg Lys
355 360 365

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Phe Thr Glu Trp Ala Tyr Gly Pro Val His Ser Ser Leu Tyr Asp Leu
370 375 380 385

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Ser Cys Ile Asp Thr Cys Glu Lys Asn Ser Val Leu Glu Val Ile Ala
390 395 400

tac agc agc agc gag acc cct aat cgc cac gac atg ctc ttg gtg gag 2025
Tyr Ser Ser Ser Glu Thr Pro Asn Arg His Asp Met Leu Leu Val Glu
405 410 415

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Pro Leu Asn Arg Leu Leu Gln Asp Lys Trp Asp Arg Phe Val Lys Arg
420 425 430

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Ile Phe Tyr Phe Asn Phe Leu Val Tyr Cys Leu Tyr Met Ile Ile Phe
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acc atg gct gcc tac tac agg ccc gtg gat ggc ttg cct ccc ttt aag 2169
Thr Met Ala Ala Tyr Tyr Arg Pro Val Asp Gly Leu Pro Pro Phe Lys
450 455 460 465

atg gaa aaa att gga gac tat ttc cga gtt act gga gag atc ctg tct 2217
Met Glu Lys Ile Gly Asp Tyr Phe Arg Val Thr Gly Glu Ile Leu Ser
470 475 480

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Val Leu Gly Gly Val Tyr Phe Phe Phe Arg Gly Ile Gln Tyr Phe Leu
485 490 495

cag agg cgg ccg tcg atg aag acc ctg ttt gtg gac agc tac agt gag 2313
 Gln Arg Arg Pro Ser Met Lys Thr Leu Phe Val Asp Ser Tyr Ser Glu
 500 505 510

atg ctt ttc ttt ctg cag tca ctg ttc atg ctg gcc acc gtg gtg ctg 2361
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 Tyr Phe Ser His Leu Lys Glu Tyr Val Ala Ser Met Val Phe Ser Leu
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gcc ttg ggc tgg acc aac atg ctc tac tac acc cgc ggt ttc cag cag 2457
 Ala Leu Gly Trp Thr Asn Met Leu Tyr Tyr Thr Arg Gly Phe Gln Gln
 550 555 560

atg ggc atc tat gcc gtc atg ata gag aag atg atc ctg aga gac ctg 2505
 Met Gly Ile Tyr Ala Val Met Ile Glu Lys Met Ile Leu Arg Asp Leu
 565 570 575

tgc cgt ttc atg ttt gtc tac atc gtc ttc ttg ttc ggg ttt tcc aca 2553
 Cys Arg Phe Met Phe Val Tyr Ile Val Phe Leu Phe Gly Phe Ser Thr
 580 585 590

gcg gtg gtg acg ctg att gaa gac ggg aag aat gac tcc ctg ccg tct 2601
 Ala Val Val Thr Leu Ile Glu Asp Gly Lys Asn Asp Ser Leu Pro Ser
 595 600 605

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 Glu Ser Thr Ser His Arg Trp Arg Gly Pro Ala Cys Arg Pro Pro Asp
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agc tcc tac aac agc ctg tac tcc acc tgc ctg gag ctg ttc aag ttc 2697
 Ser Ser Tyr Asn Ser Leu Tyr Ser Thr Cys Leu Glu Leu Phe Lys Phe
 630 635 640

acc atc ggc atg ggc gac ctg gag ttc act gag aac tat gac ttc aag 2745
Thr Ile Gly Met Gly Asp Leu Glu Phe Thr Glu Asn Tyr Asp Phe Lys
645 650 655

gct gtc ttc atc atc ctg ctg ctg gcc tat gta att ctc acc tac atc 2793
Ala Val Phe Ile Ile Leu Leu Leu Ala Tyr Val Ile Leu Thr Tyr Ile
660 665 670

ctc ctg ctc aac atg ctc atc gcc ctc atg ggt gag act gtc aac aag 2841
Leu Leu Leu Asn Met Leu Ile Ala Leu Met Gly Glu Thr Val Asn Lys
675 680 685

atc gca cag gag agc aag aac atc tgg aag ctg cag aga gcc atc acc 2889
Ile Ala Gln Glu Ser Lys Asn Ile Trp Lys Leu Gln Arg Ala Ile Thr
690 695 700 705

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710 715 720

cgc tca ggc aag ctg ctg cag gtg ggg tac aca cct gat ggc aag gac 2985
Arg Ser Gly Lys Leu Leu Gln Val Gly Tyr Thr Pro Asp Gly Lys Asp
725 730 735

gac tac cgg tgg tgc ttc agg gtg gac gag gtg aac tgg acc acc tgg 3033
Asp Tyr Arg Trp Cys Phe Arg Val Asp Glu Val Asn Trp Thr Thr Trp
740 745 750

aac acc aac gtg ggc atc atc aac gaa gac ccg ggc aac tgt gag ggc 3081
Asn Thr Asn Val Gly Ile Ile Asn Glu Asp Pro Gly Asn Cys Glu Gly
755 760 765

gtc aag cgc acc ctg agc ttc tcc ctg cgg tca agc aga gtt tca ggc 3129
Val Lys Arg Thr Leu Ser Phe Ser Leu Arg Ser Ser Arg Val Ser Gly
770 775 780 785

aga cac tgg aag aac ttt gcc ctg gtc ccc ctt tta aga gag gca agt 3177
Arg His Trp Lys Asn Phe Ala Leu Val Pro Leu Leu Arg Glu Ala Ser
790 795 800

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Ala Arg Asp Arg Gln Ser Ala Gln Pro Glu Glu Val Tyr Leu Arg Gln
805 810 815

ttt tca ggg tct ctg aag cca gag gac gct gag gtc ttc aag agt cct 3273
Phe Ser Gly Ser Leu Lys Pro Glu Asp Ala Glu Val Phe Lys Ser Pro
820 825 830

gcc gct tcc ggg gag aag tga ggacgtcacg cagacagcac tgtcaacact 3324
Ala Ala Ser Gly Glu Lys
835

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35 40 45

Leu Phe Gly Lys Gly Asp Ser Glu Glu Ala Phe Pro Val Asp Cys Pro
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His Glu Glu Gly Glu Leu Asp Ser Cys Pro Thr Ile Thr Val Ser Pro
65 70 75 80

Val Ile Thr Ile Gln Arg Pro Gly Asp Gly Pro Thr Gly Ala Arg Leu
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Leu Ser Gln Asp Ser Val Ala Ala Ser Thr Glu Lys Thr Leu Arg Leu
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Tyr Asp Arg Arg Ser Ile Phe Glu Ala Val Ala Gln Asn Asn Cys Gln
115 120 125

Asp Leu Glu Ser Leu Leu Leu Phe Leu Gln Lys Ser Lys Lys His Leu
130 135 140

Thr Asp Asn Glu Phe Lys Asp Pro Glu Thr Gly Lys Thr Cys Leu Leu
145 150 155 160

Lys Ala Met Leu Asn Leu His Asp Gly Gln Asn Thr Thr Ile Pro Leu
165 170 175

Leu Leu Glu Ile Ala Arg Gln Thr Asp Ser Leu Lys Glu Leu Val Asn
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Ala Ser Tyr Thr Asp Ser Tyr Tyr Lys Gly Gln Thr Ala Leu His Ile
195 200 205

Ala Ile Glu Arg Arg Asn Met Ala Leu Val Thr Leu Leu Val Glu Asn
210 215 220

Gly Ala Asp Val Gln Ala Ala Ala His Gly Asp Phe Phe Lys Lys Thr
225 230 235 240

Lys Gly Arg Pro Gly Phe Tyr Phe Gly Glu Leu Pro Leu Ser Leu Ala
245 250 255

Ala Cys Thr Asn Gln Leu Gly Ile Val Lys Phe Leu Leu Gln Asn Ser
260 265 270

Trp Gln Thr Ala Asp Ile Ser Ala Arg Asp Ser Val Gly Asn Thr Val
275 280 285

Leu His Ala Leu Val Glu Val Ala Asp Asn Thr Ala Asp Asn Thr Lys
290 295 300

Phe Val Thr Ser Met Tyr Asn Glu Ile Leu Ile Leu Gly Ala Lys Leu
305 310 315 320

His Pro Thr Leu Lys Leu Glu Glu Leu Thr Asn Lys Lys Gly Met Thr
325 330 335

Pro Leu Ala Leu Ala Ala Gly Thr Gly Lys Ile Gly Val Leu Ala Tyr
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Lys Phe Thr Glu Trp Ala Tyr Gly Pro Val His Ser Ser Leu Tyr Asp
370 375 380

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385 390 395 400

Ala Tyr Ser Ser Ser Glu Thr Pro Asn Arg His Asp Met Leu Leu Val
405 410 415

Glu Pro Leu Asn Arg Leu Leu Gln Asp Lys Trp Asp Arg Phe Val Lys
420 425 430

Arg Ile Phe Tyr Phe Asn Phe Leu Val Tyr Cys Leu Tyr Met Ile Ile
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Phe Thr Met Ala Ala Tyr Tyr Arg Pro Val Asp Gly Leu Pro Pro Phe
450 455 460

Lys Met Glu Lys Ile Gly Asp Tyr Phe Arg Val Thr Gly Glu Ile Leu
465 470 475 480

Ser Val Leu Gly Gly Val Tyr Phe Phe Phe Arg Gly Ile Gln Tyr Phe
485 490 495

Leu Gln Arg Arg Pro Ser Met Lys Thr Leu Phe Val Asp Ser Tyr Ser
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Glu Met Leu Phe Phe Leu Gln Ser Leu Phe Met Leu Ala Thr Val Val
515 520 525

Leu Tyr Phe Ser His Leu Lys Glu Tyr Val Ala Ser Met Val Phe Ser
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Gln Met Gly Ile Tyr Ala Val Met Ile Glu Lys Met Ile Leu Arg Asp
565 570 575

Leu Cys Arg Phe Met Phe Val Tyr Ile Val Phe Leu Phe Gly Phe Ser
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Thr Ala Val Val Thr Leu Ile Glu Asp Gly Lys Asn Asp Ser Leu Pro
595 600 605

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Asp Ser Ser Tyr Asn Ser Leu Tyr Ser Thr Cys Leu Glu Leu Phe Lys
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Lys Ala Val Phe Ile Ile Leu Leu Leu Ala Tyr Val Ile Leu Thr Tyr
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675 680 685

Lys Ile Ala Gln Glu Ser Lys Asn Ile Trp Lys Leu Gln Arg Ala Ile
690 695 700

Thr Ile Leu Asp Thr Glu Lys Ser Phe Leu Lys Cys Met Arg Lys Ala
705 710 715 720

Phe Arg Ser Gly Lys Leu Leu Gln Val Gly Tyr Thr Pro Asp Gly Lys
725 730 735

Asp Asp Tyr Arg Trp Cys Phe Arg Val Asp Glu Val Asn Trp Thr Thr
740 745 750

Trp Asn Thr Asn Val Gly Ile Ile Asn Glu Asp Pro Gly Asn Cys Glu
755 760 765

Gly Val Lys Arg Thr Leu Ser Phe Ser Leu Arg Ser Ser Arg Val Ser
770 775 780

Gly Arg His Trp Lys Asn Phe Ala Leu Val Pro Leu Leu Arg Glu Ala
785 790 795 800

Ser Ala Arg Asp Arg Gln Ser Ala Gln Pro Glu Glu Val Tyr Leu Arg
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Pro Ala Ala Ser Gly Glu Lys
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35 40 45

Leu Phe Gly Lys Gly Asp Ser Glu Glu Ala Ser Pro Leu Asp Cys Pro
50 55 60

Tyr Glu Glu Gly Gly Leu Ala Ser Cys Pro Ile Ile Thr Val Ser Ser
65 70 75 80

Val Leu Thr Ile Gln Arg Pro Gly Asp Gly Pro Ala Ser Val Arg Pro
85 90 95

Ser Ser Gln Asp Ser Val Ser Ala Gly Glu Lys Pro Pro Arg Leu Tyr
100 105 110

Asp Arg Arg Ser Ile Phe Asp Ala Val Ala Gln Ser Asn Cys Gln Glu
115 120 125

Leu Glu Ser Leu Leu Pro Phe Leu Gln Arg Ser Lys Lys Arg Leu Thr
130 135 140

Asp Ser Glu Phe Lys Asp Pro Glu Thr Gly Lys Thr Cys Leu Leu Lys
145 150 155 160

Ala Met Leu Asn Leu His Asn Gly Gln Asn Asp Thr Ile Ala Leu Leu
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Leu Asp Val Ala Arg Lys Thr Asp Ser Leu Lys Gln Phe Val Asn Ala
180 185 190

Ser Tyr Thr Asp Ser Tyr Tyr Lys Gly Gln Thr Ala Leu His Ile Ala
195 200 205

Ile Glu Arg Arg Asn Met Thr Leu Val Thr Leu Leu Val Glu Asn Gly
210 215 220

Ala Asp Val Gln Ala Ala Ala Asn Gly Asp Phe Phe Lys Lys Thr Lys
225 230 235 240

Gly Arg Pro Gly Phe Tyr Phe Gly Glu Leu Pro Leu Ser Leu Ala Ala
245 250 255

Cys Thr Asn Gln Leu Ala Ile Val Lys Phe Leu Leu Gln Asn Ser Trp
260 265 270

Gln Pro Ala Asp Ile Ser Ala Arg Asp Ser Val Gly Asn Thr Val Leu
275 280 285

His Ala Leu Val Glu Val Ala Asp Asn Thr Val Asp Asn Thr Lys Phe
290 295 300

Val Thr Ser Met Tyr Asn Glu Ile Leu Ile Leu Gly Ala Lys Leu His
305 310 315 320

Pro Thr Leu Lys Leu Glu Glu Ile Thr Asn Arg Lys Gly Leu Thr Pro
325 330 335

Leu Ala Leu Ala Ala Ser Ser Gly Lys Ile Gly Val Leu Ala Tyr Ile
340 345 350

Leu Gln Arg Glu Ile His Glu Pro Glu Cys Arg His Leu Ser Arg Lys
355 360 365

Phe Thr Glu Trp Ala Tyr Gly Pro Val His Ser Ser Leu Tyr Asp Leu
370 375 380

Ser Cys Ile Asp Thr Cys Glu Lys Asn Ser Val Leu Glu Val Ile Ala
385 390 395 400

Tyr Ser Ser Ser Glu Thr Pro Asn Arg His Asp Met Leu Leu Val Glu
405 410 415

Pro Leu Asn Arg Leu Leu Gln Asp Lys Trp Asp Arg Phe Val Lys Arg
420 425 430

Ile Phe Tyr Phe Asn Phe Phe Val Tyr Cys Leu Tyr Met Ile Ile Phe
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Thr Ala Ala Ala Tyr Tyr Arg Pro Val Glu Gly Leu Pro Pro Tyr Lys
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Leu Lys Asn Thr Val Gly Asp Tyr Phe Arg Val Thr Gly Glu Ile Leu
465 470 475 480

Ser Val Ser Gly Gly Val Tyr Phe Phe Phe Arg Gly Ile Gln Tyr Phe
485 490 495

Leu Gln Arg Arg Pro Ser Leu Lys Ser Leu Phe Val Asp Ser Tyr Ser
500 505 510

Glu Ile Leu Phe Phe Val Gln Ser Leu Phe Met Leu Val Ser Val Val
515 520 525

Leu Tyr Phe Ser Gln Arg Lys Glu Tyr Val Ala Ser Met Val Phe Ser
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Gln Met Gly Ile Tyr Ala Val Met Ile Glu Lys Met Ile Leu Arg Asp
565 570 575

Leu Cys Arg Phe Met Phe Val Tyr Leu Val Phe Leu Phe Gly Phe Ser
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595 600 605

Met Glu Ser Thr Pro His Lys Cys Arg Gly Ser Ala Cys Lys Pro Gly
610 615 620

Asn Ser Tyr Asn Ser Leu Tyr Ser Thr Cys Leu Glu Leu Phe Lys Phe
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Ala Val Phe Ile Ile Leu Leu Leu Ala Tyr Val Ile Leu Thr Tyr Ile
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Leu Leu Leu Asn Met Leu Ile Ala Leu Met Gly Glu Thr Val Asn Lys
675 680 685

Ile Ala Gln Glu Ser Lys Asn Ile Trp Lys Leu Gln Arg Ala Ile Thr
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Ile Leu Asp Thr Glu Lys Ser Phe Leu Lys Cys Met Arg Lys Ala Phe
705 710 715 720

Arg Ser Gly Lys Leu Leu Gln Val Gly Phe Thr Pro Asp Gly Lys Asp
725 730 735

Asp Tyr Arg Trp Cys Phe Arg Val Asp Glu Val Asn Trp Thr Thr Trp
740 745 750

Asn Thr Asn Val Gly Ile Ile Asn Glu Asp Pro Gly Asn Cys Glu Gly
755 760 765

Val Lys Arg Thr Leu Ser Phe Ser Leu Arg Ser Gly Arg Val Ser Gly
770 775 780

Arg Asn Trp Lys Asn Phe Ala Leu Val Pro Leu Leu Arg Asp Ala Ser
785 790 795 800

Thr Arg Asp Arg His Ala Thr Gln Gln Glu Glu Val Gln Leu Lys His
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Asn Leu Cys Phe Gln Val Arg Glu Arg Gly Ser Cys Cys Ser Ser Arg
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gtc att aca acg gtg gct ttg aag cag ctg gca gca ctg ctg ctt gtc 856
Val Ile Thr Thr Val Ala Leu Lys Gln Leu Ala Ala Leu Leu Leu Val
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cac gtg gga ggg ggc ttc ctg gag ccc ccg ccc ctg gcc ggg ttc tgc 904
 His Val Gly Gly Gly Phe Leu Glu Pro Pro Pro Leu Ala Gly Phe Cys
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ccg ccc ccc atc ctc aaa gtc ttc aac cgg cct atc ctc ttt gac atc 1432
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Asn Met Arg Glu Phe Ile Asn Ser Pro Phe Arg Asp Ile Tyr Tyr Arg	
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Glu Leu Pro Leu Ser Leu Ala Ala Cys Thr Asn Gln Pro His Ile Val	
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Asp Ser Arg Gly Asn Thr Val Leu His Ala Leu Val Ala Ile Ala Asp	
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460 465 470

aag att ggg atc ttt cag cac atc atc cgg cgg gag gtg acg gat gag 2152
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475 480 485

gac aca cgg cac ctg tcc cgc aag tcc aag gac tgg gcc tat ggg cca 2200
Asp Thr Arg His Leu Ser Arg Lys Ser Lys Asp Trp Ala Tyr Gly Pro
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Val Tyr Ser Ser Leu Tyr Asp Leu Ser Ser Leu Asp Thr Cys Gly Glu
510 515 520

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Glu Ala Ser Val Leu Glu Ile Leu Val Tyr Asn Ser Lys Ile Glu Asn
525 530 535

cgc cac gag atg ctg gct gtg gag ccc atc aat gaa ctg ctg cgg gac 2344
Arg His Glu Met Leu Ala Val Glu Pro Ile Asn Glu Leu Leu Arg Asp
540 545 550

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Lys Trp Arg Lys Phe Gly Ala Val Ser Phe Tyr Ile Asn Val Val Ser
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tac ctg tgt gcc atg gtt atc ttc act ctc acc gcc tac tac cag ccg 2440
Tyr Leu Cys Ala Met Val Ile Phe Thr Leu Thr Ala Tyr Tyr Gln Pro
570 575 580 585

ctg gag ggc aca ccg ccg tac cct tac cgc acc acg gtg gac tac ctg 2488
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 620 625 630

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 635 640 645

gtc ctg gtg atc gtc tca gca gcc ctc tac ctg gca ggg atc gag gcc 2680
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 650 655 660 665

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 685 690 695

atc cag aag att ctc ttc aag gac ctt ttc cga ttc ctg ctc gtc tac 2824
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 715 720 725

ccg tgt gcc aac atg aag gtg tgc aat gag gac cag acc aac tgc aca 2920
Pro Cys Ala Asn Met Lys Val Cys Asn Glu Asp Gln Thr Asn Cys Thr
730 735 740 745

gtg ccc act tac ccc tcg tgc cgt gac agc gag acc ttc agc acc ttc 2968
Val Pro Thr Tyr Pro Ser Cys Arg Asp Ser Glu Thr Phe Ser Thr Phe
750 755 760

ctc ctg gac ctg ttt aag ctg acc atc ggc atg ggc gac ctg gag atg 3016
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765 770 775

ctg agc agc acc aag tac ccc gtg gtc ttc atc atc ctg ctg gtg acc 3064
Leu Ser Ser Thr Lys Tyr Pro Val Val Phe Ile Ile Leu Leu Val Thr
780 785 790

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Tyr Ile Ile Leu Thr Ser Val Leu Leu Leu Asn Met Leu Ile Ala Leu
795 800 805

atg ggc gag aca gtg ggc cag gtc tcc aag gag agc aag cac atc tgg 3160
Met Gly Glu Thr Val Gly Gln Val Ser Lys Glu Ser Lys His Ile Trp
810 815 820 825

aag ctg cag tgg gcc acc acc atc ctg gac att gag cgc tcc ttc ccc 3208
Lys Leu Gln Trp Ala Thr Thr Ile Leu Asp Ile Glu Arg Ser Phe Pro
830 835 840

gta ttc ctg agg aag gcc ttc cgc tct ggg gag atg gtc acc gtg ggc 3256
Val Phe Leu Arg Lys Ala Phe Arg Ser Gly Glu Met Val Thr Val Gly
845 850 855

aag agc tcg gac ggc act cct gac cgc agg tgg tgc ttc agg gtg gat 3304
Lys Ser Ser Asp Gly Thr Pro Asp Arg Arg Trp Cys Phe Arg Val Asp
860 865 870

gag gtg aac tgg tct cac tgg aac cag aac ttg ggc atc atc aac gag 3352
Glu Val Asn Trp Ser His Trp Asn Gln Asn Leu Gly Ile Ile Asn Glu
875 880 885

gac ccg ggc aag aat gag acc tac cag tat tat ggc ttc tcg cat acc 3400
Asp Pro Gly Lys Asn Glu Thr Tyr Gln Tyr Tyr Gly Phe Ser His Thr
890 895 900 905

gtg ggc cgc ctc cgc agg gat cgc tgg tcc tcg gtg gta ccc cgc gtg 3448
Val Gly Arg Leu Arg Arg Asp Arg Trp Ser Ser Val Val Pro Arg Val
910 915 920

gtg gaa ctg aac aag aac tcg aac ccg gac gag gtg gtg gtg cct ctg 3496
Val Glu Leu Asn Lys Asn Ser Asn Pro Asp Glu Val Val Val Pro Leu
925 930 935

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Asp Ser Met Gly Asn Pro Arg Cys Asp Gly His Gln Gln Gly Tyr Pro
940 945 950

cgc aag tgg agg act gat gac gcc ccg ctc tag ggactgcagc ccagccccag 3597
Arg Lys Trp Arg Thr Asp Asp Ala Pro Leu
955 960

cttctctgcc cactcatttc tagtccagcc gcatttcagc agtgccttct ggggtgtccc 3657

cccacaccct gctttggccc cagagggcag ggaccagtgg aggtgccagg gagggcccag 3717

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<211> 963

<212> PRT

<213> Homo sapiens

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Glu Pro Pro Pro Leu Ala Gly Phe Cys Leu Thr Pro Leu Ser Phe Pro
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Ser Glu Gly Pro Arg Ala Gly Pro Gly Glu Val Ala Glu Leu Pro Gly
100 105 110

Asp Glu Ser Gly Thr Pro Gly Gly Glu Ala Phe Pro Leu Ser Ser Leu
115 120 125

Ala Asn Leu Phe Glu Gly Glu Asp Gly Ser Leu Ser Pro Ser Pro Ala
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Asp Ala Ser Arg Pro Ala Gly Pro Gly Asp Gly Arg Pro Asn Leu Arg
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165 170 175

Leu Leu Glu Ser Thr Leu Tyr Glu Ser Ser Val Val Pro Gly Pro Lys
180 185 190

Lys Ala Pro Met Asp Ser Leu Phe Asp Tyr Gly Thr Tyr Arg His His
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Ser Ser Asp Asn Lys Arg Trp Arg Lys Lys Ile Ile Glu Lys Gln Pro
210 215 220

Gln Ser Pro Lys Ala Pro Ala Pro Gln Pro Pro Pro Ile Leu Lys Val
225 230 235 240

Phe Asn Arg Pro Ile Leu Phe Asp Ile Val Ser Arg Gly Ser Thr Ala
245 250 255

Asp Leu Asp Gly Leu Leu Pro Phe Leu Leu Thr His Lys Lys Arg Leu
260 265 270

Thr Asp Glu Glu Phe Arg Glu Pro Ser Thr Gly Lys Thr Cys Leu Pro
275 280 285

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290 295 300

Leu Leu Asp Ile Ala Glu Arg Thr Gly Asn Met Arg Glu Phe Ile Asn
305 310 315 320

Ser Pro Phe Arg Asp Ile Tyr Tyr Arg Gly Gln Thr Ala Leu His Ile
325 330 335

Ala Ile Glu Arg Arg Cys Lys His Tyr Val Glu Leu Leu Val Ala Gln
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Gly Ala Asp Val His Ala Gln Ala Arg Gly Arg Phe Phe Gln Pro Lys
355 360 365

Asp Glu Gly Gly Tyr Phe Tyr Phe Gly Glu Leu Pro Leu Ser Leu Ala
370 375 380

Ala Cys Thr Asn Gln Pro His Ile Val Asn Tyr Leu Thr Glu Asn Pro
385 390 395 400

His Lys Lys Ala Asp Met Arg Arg Gln Asp Ser Arg Gly Asn Thr Val
405 410 415

Leu His Ala Leu Val Ala Ile Ala Asp Asn Thr Arg Glu Asn Thr Lys
420 425 430

Phe Val Thr Lys Met Tyr Asp Leu Leu Leu Leu Lys Cys Ala Arg Leu
435 440 445

Phe Pro Asp Ser Asn Leu Glu Ala Val Leu Asn Asn Asp Gly Leu Ser
450 455 460

Pro Leu Met Met Ala Ala Lys Thr Gly Lys Ile Gly Ile Phe Gln His
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485 490 495

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Leu Ser Ser Leu Asp Thr Cys Gly Glu Glu Ala Ser Val Leu Glu Ile
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530 535 540

Glu Pro Ile Asn Glu Leu Leu Arg Asp Lys Trp Arg Lys Phe Gly Ala
545 550 555 560

Val Ser Phe Tyr Ile Asn Val Val Ser Tyr Leu Cys Ala Met Val Ile
565 570 575

Phe Thr Leu Thr Ala Tyr Tyr Gln Pro Leu Glu Gly Thr Pro Pro Tyr
580 585 590

Pro Tyr Arg Thr Thr Val Asp Tyr Leu Arg Leu Ala Gly Glu Val Ile
595 600 605

Thr Leu Phe Thr Gly Val Leu Phe Phe Phe Thr Asn Ile Lys Asp Leu
610 615 620

Phe Met Lys Lys Cys Pro Gly Val Asn Ser Leu Phe Ile Asp Gly Ser
625 630 635 640

Phe Gln Leu Leu Tyr Phe Ile Tyr Ser Val Leu Val Ile Val Ser Ala
645 650 655

Ala Leu Tyr Leu Ala Gly Ile Glu Ala Tyr Leu Ala Met Met Val Phe
660 665 670

Ala Leu Val Leu Gly Trp Met Asn Ala Leu Tyr Phe Thr Arg Gly Leu
675 680 685

Lys Leu Thr Gly Thr Tyr Ser Ile Met Ile Gln Lys Ile Leu Phe Lys
690 695 700

Asp Leu Phe Arg Phe Leu Leu Val Tyr Leu Leu Phe Met Ile Gly Tyr
705 710 715 720

Ala Ser Ala Leu Val Ser Leu Leu Asn Pro Cys Ala Asn Met Lys Val
725 730 735

Cys Asn Glu Asp Gln Thr Asn Cys Thr Val Pro Thr Tyr Pro Ser Cys
740 745 750

Arg Asp Ser Glu Thr Phe Ser Thr Phe Leu Leu Asp Leu Phe Lys Leu
755 760 765

Thr Ile Gly Met Gly Asp Leu Glu Met Leu Ser Ser Thr Lys Tyr Pro
770 775 780

Val Val Phe Ile Ile Leu Leu Val Thr Tyr Ile Ile Leu Thr Ser Val
785 790 795 800

Leu Leu Leu Asn Met Leu Ile Ala Leu Met Gly Glu Thr Val Gly Gln
805 810 815

Val Ser Lys Glu Ser Lys His Ile Trp Lys Leu Gln Trp Ala Thr Thr
820 825 830

Ile Leu Asp Ile Glu Arg Ser Phe Pro Val Phe Leu Arg Lys Ala Phe
835 840 845

Arg Ser Gly Glu Met Val Thr Val Gly Lys Ser Ser Asp Gly Thr Pro
850 855 860

Asp Arg Arg Trp Cys Phe Arg Val Asp Glu Val Asn Trp Ser His Trp
865 870 875 880

Asn Gln Asn Leu Gly Ile Ile Asn Glu Asp Pro Gly Lys Asn Glu Thr
885 890 895

Tyr Gln Tyr Tyr Gly Phe Ser His Thr Val Gly Arg Leu Arg Arg Asp
900 905 910

Arg Trp Ser Ser Val Val Pro Arg Val Val Glu Leu Asn Lys Asn Ser
915 920 925

Asn Pro Asp Glu Val Val Val Pro Leu Asp Ser Met Gly Asn Pro Arg
930 935 940

Cys Asp Gly His Gln Gln Gly Tyr Pro Arg Lys Trp Arg Thr Asp Asp
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<212> PRT

<213> Homo sapiens

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Lys Phe Ala Pro Gln Ile Arg Val Asn Leu Asn Tyr Arg Lys Gly Thr
50 55 60

Gly Ala Ser Gln Pro Asp Pro Asn Arg Phe Asp Arg Asp Arg Leu Phe
65 70 75 80

Asn Ala Val Ser Arg Gly Val Pro Glu Asp Leu Ala Gly Leu Pro Glu
85 90 95

Tyr Leu Ser Lys Thr Ser Lys Tyr Leu Thr Asp Ser Glu Tyr Thr Glu
100 105 110

Gly Ser Thr Gly Lys Thr Cys Leu Met Lys Ala Val Leu Asn Leu Lys
115 120 125

Asp Gly Val Asn Ala Cys Ile Leu Pro Leu Leu Gln Ile Asp Arg Asp
130 135 140

Ser Gly Asn Pro Gln Pro Leu Val Asn Ala Gln Cys Thr Asp Asp Tyr
145 150 155 160

Tyr Arg Gly His Ser Ala Leu His Ile Ala Ile Glu Lys Arg Ser Leu
165 170 175

Gln Cys Val Lys Leu Leu Val Glu Asn Gly Ala Asn Val His Ala Arg
180 185 190

Ala Cys Gly Arg Phe Phe Gln Lys Gly Gln Gly Thr Cys Phe Tyr Phe
195 200 205

Gly Glu Leu Pro Leu Ser Leu Ala Ala Cys Thr Lys Gln Trp Asp Val
210 215 220

Val Ser Tyr Leu Leu Glu Asn Pro His Gln Pro Ala Ser Leu Gln Ala
225 230 235 240

Thr Asp Ser Gln Gly Asn Thr Val Leu His Ala Leu Val Met Ile Ser
245 250 255

Asp Asn Ser Ala Glu Asn Ile Ala Leu Val Thr Ser Met Tyr Asp Gly
260 265 270

Leu Leu Gln Ala Gly Ala Arg Leu Cys Pro Thr Val Gln Leu Glu Asp
275 280 285

Ile Arg Asn Leu Gln Asp Leu Thr Pro Leu Lys Leu Ala Ala Lys Glu
290 295 300

Gly Lys Ile Glu Ile Phe Arg His Ile Leu Gln Arg Glu Phe Ser Gly
305 310 315 320

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325 330 335

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340 345 350

Ser Val Leu Glu Ile Ile Ala Phe His Cys Lys Ser Pro His Arg His
355 360 365

Arg Met Val Val Leu Glu Pro Leu Asn Lys Leu Leu Gln Ala Lys Trp
370 375 380

Asp Leu Leu Ile Pro Lys Phe Phe Leu Asn Phe Leu Cys Asn Leu Ile
385 390 395 400

Tyr Met Phe Ile Phe Thr Ala Val Ala Tyr His Gln Pro Thr Leu Lys
405 410 415

Lys Gln Ala Ala Pro His Leu Lys Ala Glu Val Gly Asn Ser Met Leu
420 425 430

Leu Thr Gly His Ile Leu Ile Leu Leu Gly Gly Ile Tyr Leu Leu Val
435 440 445

Gly Gln Leu Trp Tyr Phe Trp Arg Arg His Val Phe Ile Trp Ile Ser
450 455 460

Phe Ile Asp Ser Tyr Phe Glu Ile Leu Phe Leu Phe Gln Ala Leu Leu
465 470 475 480

Thr Val Val Ser Gln Val Leu Cys Phe Leu Ala Ile Glu Trp Tyr Leu
485 490 495

Pro Leu Leu Val Ser Ala Leu Val Leu Gly Trp Leu Asn Leu Leu Tyr
500 505 510

Tyr Thr Arg Gly Phe Gln His Thr Gly Ile Tyr Ser Val Met Ile Gln
515 520 525

Lys Val Ile Leu Arg Asp Leu Leu Arg Phe Leu Leu Ile Tyr Leu Val
530 535 540

Phe Leu Phe Gly Phe Ala Val Ala Leu Val Ser Leu Ser Gln Glu Ala
545 550 555 560

Trp Arg Pro Glu Ala Pro Thr Gly Pro Asn Ala Thr Glu Ser Val Gln
565 570 575

Pro Met Glu Gly Gln Glu Asp Glu Gly Asn Gly Ala Gln Tyr Arg Gly
580 585 590

Ile Leu Glu Ala Ser Leu Glu Leu Phe Lys Phe Thr Ile Gly Met Gly
595 600 605

Glu Leu Ala Phe Gln Glu Gln Leu His Phe Arg Gly Met Val Leu Leu
610 615 620

Leu Leu Leu Ala Tyr Val Leu Leu Thr Tyr Ile Leu Leu Leu Asn Met
625 630 635 640

Leu Ile Ala Leu Met Ser Glu Thr Val Asn Ser Val Ala Thr Asp Ser
645 650 655

Trp Ser Ile Trp Lys Leu Gln Lys Ala Ile Ser Val Leu Glu Met Glu
660 665 670

Asn Gly Tyr Trp Trp Cys Arg Lys Lys Gln Arg Ala Gly Val Met Leu
675 680 685

Thr Val Gly Thr Lys Pro Asp Gly Ser Pro Asp Glu Arg Trp Cys Phe
690 695 700

Arg Val Glu Glu Val Asn Trp Ala Ser Trp Glu Gln Thr Leu Pro Thr
705 710 715 720

Leu Cys Glu Asp Pro Ser Gly Ala Gly Val Pro Arg Thr Leu Glu Asn
725 730 735

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Glu Asn Tyr Val Pro Val Gln Leu Leu Gln Ser Asn
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<212> DNA

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<220>

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<212> DNA

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<223> Description of Artificial Sequence: Primer

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<223> Description of Artificial Sequence: Primer

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<210> 11

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<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Primer

<400> 11

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<210> 12

<211> 20

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Primer

<400> 12

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<210> 13

<211> 20

<212> DNA

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<220>

<223> Description of Artificial Sequence: Primer

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<210> 14

<211> 19

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Primer

<400> 14

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<210> 15

<211> 20

<212> DNA

<213> Artificial Sequence

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<223> Description of Artificial Sequence: Primer

<400> 15

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<210> 16

<211> 21

<212> DNA

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<223> Description of Artificial Sequence: Primer

<400> 16

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<210> 17

<211> 20

<212> DNA

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<210> 18

<211> 21

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<210> 19

<211> 43

<212> DNA

<213> Artificial Sequence

<220>

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<210> 20

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<212> DNA

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<220>

<223> Description of Artificial Sequence: Primer

<400> 20

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<210> 21

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<210> 22

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<212> DNA

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<210> 23

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<211> 32

<212> DNA

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<400> 24

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<210> 25

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Asp Ser Glu Glu Ala Ser Cys

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<223> Description of Artificial Sequence: Synthetic
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Val Pro Gly Glu Lys
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<213> Artificial Sequence

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<223> Description of Artificial Sequence: Primer

<400> 30

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<223> Description of Artificial Sequence: Primer

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<210> 34

<211> 23

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Primer

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<210> 35

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<223> Description of Artificial Sequence: Primer

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<210> 36

<211> 32

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<210> 37

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<220>

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<210> 38

<211> 18

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Primer

<400> 38

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18

<210> 39

<211> 20

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Primer

<400> 39

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<210> 40

<211> 20

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Primer

<400> 40

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20

INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 99/09284

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C12N15/12 C07K14/705 C12N15/85 C12N5/10 C07K16/28

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C12N C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CATERINA, M.J. ET AL.: "The capsaicin receptor: a heat-activated ion channel in the pain pathway" NATURE, vol. 389, no. 6653, 23 October 1997 (1997-10-23), pages 816-824, XP002075020 cited in the application abstract page 819; figures 5A,C page 820, column 2, line 13 -page 821, column 1, line 29 page 823, column 2, line 13 - line 14 page 817, column 2, line 12 -page 820, column 1, line 21 page 823, column 2, line 19 -page 824, column 1, line 5	1-3,6,9, 14-16, 45-47
X		26
A		4,5,7,8, 10-13, 17-25, 48-51
	--/--	

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

11 April 2000

Date of mailing of the international search report

09/05/2000

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
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Fax: (+31-70) 340-3016

Authorized officer

Fuchs, U

INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 99/09284

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>page 817; figures 2A-C page 818; figures 3A-F ----</p> <p>EMBL Database, Heidelberg, FRG Emest2 accession number AA700891 22 December 1997 Hillier, L. ET AL.: "zj40d01.s1 Soares fetal liver spleen INFLS S1 Homo sapiens cDNA clone 452737 3'" XP002135284 the whole document</p>	6,7
X	<p>EMBL Database, Heidelberg, FRG Emest6 accession number AI089668 19 August 1998 NCI/NINDS-CGAP: "qa10f06.x1 NCI_CGAP_Brn23 Homo sapiens cDNA clone IMAGE:1686371 3'" XP002135285 the whole document</p>	6,8
X	<p>BIRO, T. ET AL.: "Recent Advances in Understanding of Vanilloid Receptors: A Therapeutic Target for Treatment of Pain and Inflammation in Skin" JOURNAL OF INVESTIGATIVE DERMATOLOGY SYMPOSIUM PROCEEDINGS, vol. 2, no. 1, August 1997 (1997-08), pages 56-60, XP002075021</p>	48,49
A	<p>abstract page 57; table 1 page 58, column 1, line 8 -column 2, line 16</p>	50,51
P,X	<p>WO 99 37675 A (THE REGENTS OF THE UNIVERSITY OF CALIFORNIA) 29 July 1999 (1999-07-29) abstract page 1, line 1 -page 3, line 30 SEQ ID NOS: 33 and 34 page 100 -page 106 page 58 -page 59; claims 1,24-6,8-14,19 ----- -/--</p>	<p>1,2,4,6, 7,9,10, 12,14, 15,23, 24,26, 45,46, 48,50</p>

INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 99/09284

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P, X	<p>EP 0 943 683 A (SMITHKLINE BEECHAM PLC) 22 September 1999 (1999-09-22)</p> <p>abstract page 2, line 1 - line 31 SEQ ID NOS: 1 and 2 page 14-16 page 36 -page 37; claims 1-14 -----</p>	<p>1,2,4,6, 7,9,10, 12,14, 15,23, 24,26, 45,46</p>

INTERNATIONAL SEARCH REPORT

International application No.

PCT/EP 99/09284

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☒ Claims Nos.: 27-45
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
see FURTHER INFORMATION sheet PCT/ISA/210
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International Application No. PCT/EP 99 09284

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 27-45

Claims 27 - 45 refer to a compound which modulates human vanilloid receptor activity without giving a true technical characterization. Moreover, except two compounds already known in the prior art, no such compounds are defined in the application. In consequence, the scopes of said claims are ambiguous and vague, and their subject matter is not sufficiently disclosed and supported (Art. 5 and 6 PCT).

No search can be carried out for such purely speculative claims whose wording is, in fact, a mere recitation of the result to be achieved.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/EP 99/09284

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9937675 A	29-07-1999	AU 2466799 A	09-08-1999
		AU 9115698 A	08-03-1999
		WO 9909140 A	25-02-1999
EP 0943683 A	22-09-1999	JP 11279196 A	12-10-1999